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ATMOSPHERIC AMMONIUM DEPOSITION AND THE NUTRITIONAL BALANCE OF TERRESTRIAL ECOSYSTEMS

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VOORWOORD

Met het tot stand komen van dit proefschrift komt voor mij een einde aan een langdurig verblijf op de afdeling Aquatische Oecologie. Het onderzoek, waaraan ik als student in 1984 deelnam, bleek achteraf het begin te vormen van deze uitgebreide studie naar de effecten van 'zure regen' op bos en heide. Gedurende deze periode veranderde met de naam van de afdeling ook de aard van het onderzoek. De puur aquatische studies die aanvankelijk werden uitgevoerd op de afdeling Aquatische Oecologie gingen geleidelijk over in het hier beschreven onderzoek aan terrestische systemen, eerst binnen de afdeling Aquatische Oecologie en Biogeologie, later in de werkgroep Milieubiologie.

Bij alle onderzoeken waaraan ik op de afdeling deelnam heb ik nauw samengewerkt met Jan Roelofs. Voor deze prettige samenwerking en alle hulp de afgelopen jaren wil ik hem hartelijk bedanken. Ook Dries Boxman en Harrie van Dijk, die samen met mij de 'droge poot' van deze waterige afdeling vormden, hebben me tijdens dit promotieonderzoek steeds bijgestaan met goede adviezen, ideeën en kritiek waarvoor ik hen zeer dankbaar ben. Prof. den Hartog bedank ik voor zijn kritisch commentaar ten aanzien van het manuscript. Naast hen wil ik echter ook alle overige (ex-)medewerkers van de afdeling bedanken voor hun hulp en de prettige en gezellige samenwerking.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

At present air pollution is considered one of the major causes of environmental changes accounting for the deterioration of aquatic as well as terrestrial ecosystems. The first acid rain related problems have been recognized two to three decades ago and concerned freshwater acidification and the reduction of fish populations in lakes of Scandinavia and North America. These observations were attributed to the acidifying effects of sulphur and nitrogen oxides. In the early eighties large scale decline of forests in the Federal Republic of Germany was observed. Some scientists considered that this decline was caused by aluminium toxicity as a result of soil acidification (Ulrich, 1983) while others ascribed the decline mainly to ozone induced damage to the canopy (Krause *et al.*, 1984).

Only in the late seventies environmental acidification was recognized as a problem in the Netherlands. Changes in desmid and diatom assemblages, macrophytes and macrofauna composition and fish populations of surface waters were attributed to the acidifying impact of atmospheric deposition (Van Dam *et al.*, 1981; Roelofs, 1983; Schuurkes, 1987; Van Dam, 1987; Leuven, 1988; Arts, 1990). Particularly, small shallow lakes, ponds and moorland pools in the Southern and Eastern parts of the Netherlands appeared to be very susceptible to acidification. At the same time it became clear that also the ongoing decline of Dutch forests could be attributed to the increased input of atmospheric pollutants. However, the damage pattern of forests did not coincide with the high-emission patterns of acidifying sulphur and nitrogen oxides nor with those of ozone. The most severely affected stands appeared to be situated in the South-eastern part of the Netherlands (Janssen, 1982). Particularly in this area high NH_3-emissions were registered. Gradually, it became evident that the specific air pollutants in The Netherlands deviated from those in many other parts of Europe and North America. Many damage symptoms of aquatic as well as of terrestrial ecosystems were found to be related to the enhanced input of reduced nitrogen compounds.

In the Netherlands the ecosystems most susceptible to air pollution, such as the earlier mentioned soft waters, heathlands and coniferous forests, are mainly situated on the nutrient poor, acid sandy soils in the Southern and Eastern part of the country. Growth of species of heathlands and forests is usually nitrogen limited. The increased nitrogen availability due the enhanced nitrogen deposition can account for the changed species composition of these ecosystems (Roelofs *et al.*, 1984; Ellenberg, 1985) or may cause 'nitrogen over-load' and consequently account for the forest decline in Europe (Skeffington & Wilson, 1988; Aber *et al.*, 1989).

One of the ecosystems this thesis deals with are heathlands. Dutch heathlands are relics of an old agricultural system ('potstal' system). This system was based on the constant removal of nutrients from the heathlands by sod cutting; the sods were used as fertilizers on agricultural land. In the beginning of this century this system had been abandoned due to the introduction of artificial fertilizer and most heathlands were transformed into arable land or planted with trees. A lower degree of management (sod-cutting and grazing) threatens the remaining area of these dwarfshrub-dominated systems. Nowadays in large areas of managed heathlands the dwarfshrubs have been replaced by grasses. This problem increased during the last decades and has been attributed to the increased nutrient availability as a result of air pollution (Berendse & Aerts, 1984; Roelofs, 1986; Heil *et al.*, 1988; Aerts, 1989). Apart from the dwarfshrub species, many herbaceous species belonging to the heathland vegetation are becoming rare and species diversity has declined dramatically (Van Dam et al.., 1986). According to Van der Eerden *et al.* (1990) these heathland species, particularly those of the Violion caninae alliance, are probably even more sensitive to atmospheric deposition than heather species.

Coniferous forests form the second ecosystem discussed in this thesis. The Netherlands do not belong to the natural distribution area of the coniferous tree species investigated. The largest part of coniferous forest stands have been planted on former heathlands as growth of these species was assumed to be well suited to the Dutch climate and relatively nutrient poor soil conditions. In the first half of this century Scots pine (*Pinus sylvestris* L.) was introduced on a large scale and this species now covers 62 % of the coniferous forest area and 40 % of all forests in the Netherlands. Some decades later, more productive species such as Corsican pine (*Pinus nigra* var. *maritima* (Ait.) Melville) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) were introduced. They occupy a much smaller area than Scots pine (each 6 % of the Dutch forested area). The annual national survey that the State Forest Service has carried out since 1984 in Dutch forests revealed that the percentage vital Scots pine trees increased from 34 in 1984 to 56 % in 1991 whereas that of Corsican pine and Douglas fir decreased from 57 and 50 % in 1984 to 23 and 8 % in 1991 respectively (State Forest Service, 1984; Informatie en Kenniscentrum NBLF, 1991). Besides the generally observed decreased vitality of forest stands a changed species composition of the understorey vegetation can often be noticed. Generally, species adapted to nitrogen poor conditions are replaced by a more nitrophilous undergrowth vegetation (Falkengren-Grerup, 1986).

Another part of the former heathland soils has been reclaimed for agricultural purposes. In the sixties many intensive livestock farms have been set up on these nutrient poor, sandy soils, leading to a situation where protected nature reserves are surrounded by NH_3-emitting farms and an increasing area of liquid-manured arable land.

The local character of NH_3-emission sources and the assumption that the aerial transport of reduced nitrogen compounds was limited, initially hampered the general acceptation of their role as atmospheric air pollutants. However, not long before the start of this research in 1984 their contribution to the acid rain problem in the Netherlands had been recognized (Van Breemen *et al.*, 1982).

According to Van der Eerden (1992) exposure of plants to high ammonia concentrations can lead to direct injuries as well as increased stress sensitivity. Fumigation experiments proved that heathland species are relatively sensitive to gaseous ammonia and that also young Douglas fir and Scots pine appear to be susceptible to increased ammonia concentrations. High concentrations of atmospheric nitrogen have been associated with many secondary stress factors such as increased sensitivity to drought, frost, fungal diseases and insect attacks (Nihlgård, 1985). In the above ground vegetation reduced nitrogen compounds can be exchanged for cations causing cation leaching in heathland species as well as coniferous tree species (Miller *et al.*, 1976; Bobbink *et al.*, 1992). Furthermore, tree canopies appear to be very effective scavengers of dry deposition resulting in high ammonium sulphate fluxes to the forest soil in wet periods (Van Breemen *et al.*, 1982). Van Breemen *et al.* (1987) have demonstrated that even in acid forest soils atmospheric ammonium can be quickly nitrified and that this nitrification can be a major source of soil acidification and subsequent leaching of nitrate. Moreover, soil acidification may lead to aluminium mobilization and the depletion of base cations. Increased aluminium concentrations are harmful to the fine root systems and lead to a decrease of mycorhizal infection (Ulrich, 1983). However, there are also forest soils in which nitrification is inhibited; this leads to increasing levels of ammonium in the top soil layer (Kriebitzsch, 1979) whereas base cations are lost to deeper layers. Increased ammonium concentrations adversely affect root/shoot, fine root/coarse root ratios and mycorhizal infection of the plant roots (Boxman & Roelofs, 1988; Van Dijk *et al.*, 1990).

In both soil types the enhanced ammonium deposition levels are expected to change the chemical soil composition which in turn may affect the vegetation. Field data to provide causal relations between ammonium fluxes and forest damage as well as experimental studies on dose-effect relations are necessary to support these hypotheses. Moreover, governmental policy needs this information to take measures against the progressive environmental deterioration.

In this thesis the decline of Dutch coniferous forests and heathlands has been studied in relation to atmospheric ammonium deposition. The main objective of this research was to find out in what way and to what extent high ammonium deposition levels affect the nutritional balance of these ecosystems. In order to answer these major questions several correlative field studies and various types of experiments have been conducted.

Chapter 2 deals with a study on the deposition of atmospheric pollutants in forested areas severely affected by NH_3 emissions. Bulk and throughfall fluxes at various distances from chicken farms and manure-dressed arable land have been estimated. Soil and needles from a number of Corsican pine stands in the South-eastern part of the Netherlands have been analyzed. Ecophysiological experiments have been carried out in order to estimate whether ammonium deposition stimulates canopy leaching. Chapters 3, 4 and 5 deal with a nation-wide investigation in Dutch coniferous forest stands. In chapter 3 the qualitative as well as the quantitative aspects of atmospheric deposition in coniferous forest stands are discussed. Bulk and throughfall fluxes in stands of Douglas fir, Corsican pine and Scots pine have been collected during 18 months. Spatial variation has been studied by comparing the chemical composition of deposition in forests of four regions. Chapter 4 deals with the impact of atmospheric deposition on the chemical soil composition and in chapter 5 the nutritional balance in needles of the three coniferous tree species has been studied in relation to throughfall fluxes as well as soil composition. In chapter 6 the effects of ammonium deposition on the nutritional balance of forest soils has been studied in more detail by the use of soil percolation columns. In these experiments forest soils with different nitrification rates have been infiltrated with an ammonium solution in a staged gradient in order to estimate the critical load of the ammonium deposition. Chapters 7 and 8 both focus on threatened plants species of Dutch heathlands. In chapter 7 the distribution of several endangered herbaceous heathland species has been studied in relation to the chemical soil composition. Chapter 8 deals with experiments conducted with *Thymus serpyllum* L. as a representative of the endangered species, to investigate in what way the decline of these species can be attributed to the enhanced ammonium deposition. Finally chapter 9 summarizes, discusses and evaluates the most important results of chapters 2 to 8.

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CHAPTER 2

THE EFFECT OF AIR-BORNE AMMONIUM SULPHATE ON *PINUS NIGRA* VAR. *MARITIMA* IN THE NETHERLANDS

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ABSTRACT

As a result of air pollution, considerable deposition of ammonium sulphate occurs on vegetation and soil in the vicinity of chicken farms and fields dressed with animal slurry. A clear relation exists between this ammonium sulphate deposition and the distance to certain agricultural activities. Field investigations and ecophysiological experiments both show that the needles of *Pinus nigra* var. *maritima* (Ait.) Melville take up ammonium and excrete potassium, magnesium and calcium. This often results in potassium and/or magnesium deficiencies and may lead to premature shedding of needles. The high levels of nitrogen in the needles are strongly correlated to fungal diseases.

Whether the observed cation leaching will result in disturbed nutrient budgets depends mainly on soil conditions. Leaching of K, Mg and Ca from the soil, caused by ammonium sulphate, may further inhibit nutrient uptake.

Field investigations show a clear correlation between increased ratios of NH_4 to K, Mg and Ca in the soil solution and the damage to pine trees.

INTRODUCTION

Recent publications concerning forest damage in Western-Europe have expressed considerable alarm, particularly in Germany where the vitality of forests has decreased rapidly. Air pollution is generally believed to be the main cause. Ulrich (1983) ascribes the phenomena mainly to soil acidification as a result of acid or acidifying substances from the atmosphere. The high Al/Ca ratio resulting from dissolution of aluminium by mineral acids and from leaching of calcium is harmful to the root system. Krause *et al.* (1984), however, believe that the observed nutrient deficiencies and concomitant damage to the trees can be ascribed mainly to leaching as a result of ozone damage to the leaves. In the Netherlands the condition of pine forests is often poor. In 1983 a nation-wide investigation, carried out by the Dutch State Forest Service (Den Boer & Bastiaens, 1983), showed that 7 to 38 % of all trees in *Pinus sylvestris* L. forests had less than one complete year-class of needles. In *Pseudotsuga menziesii* (Mirb.) Franco forests, 29-70 % of the trees had less than 50 % of the leaves considered normal for this climatic zone. 64 % of the unhealthy *Pinus sylvestris* forests and 41 % of the unhealthy *Pseudotsuga menziesil* forests showed grey-green or yellow-green discoloured needles.

Janssen (1982) and Hunger (1978) noticed a relation between intensive stockbreeding and the condition of pine trees.

On an average the total deposition of acid and potential acidifying substances in the Netherlands is ± 6 kmol equivalent $H^+.ha^{-1}.yr^{-1}$ of which ±80 % as $(NH_4)_2SO_4$

(Van Aalst, 1984). Van Breemen *et al.* (1982) and Van Breemen & Jordens (1983) state that ammonia from manure reacts with sulphur dioxide from the atmosphere, causing wet and dry deposition of large amounts of ammonium sulphate on the trees and soil (Tjepkema & Cartica, 1981). In a woodland near Warnsveld nitrification of the ammonium caused a marked pH decrease of the soil (temporarily down to pH 2.8).

In the present study precipitation analyses have been carried out at various distances from chicken farms and fields dressed with animal slurry. The chemical compositions of open field precipitation and forest canopy throughfall have been compared.

In the South-east of the Netherlands soil and needle analyses have been carried out on *Pinus nigra* var. *maritima* (Ait.) Melville at 58 locations and the results were correlated with the condition of the trees. Acid and artificially buffered soils were incubated after adding various amounts of ammonium sulphate to investigate nitrification processes. Eco-physiological experiments were carried out in order to estimate the leaching of nutrients from needles caused by ammonium sulphate.

MATERIALS AND METHODS

During 1983, fortnightly precipitation was collected and analysed, using black polyethylene bottles (1 l) and funnels (ø 10 cm), containing 1 ml of a 200 $mg.l^{-1}$ $HgCl_2$ fixing solution. In the spring and autumn 58 locations with *Pinus nigra* var. *maritima* were visited. At each location soil samples were taken, consisting of six subsamples of the mineral sandy soil, taken just below the litter layer at a depth of 15 cm, using a brass tube (length 15 cm, ø 18 cm). The samples were transported to the laboratory in a refrigerated container.

Needles were collected from each year class from trees between 30 and 50 years old. The vitality was determined from the number of year-classes of the needles. Trees with 2-3 year classes were estimated as healthy, those with 1-2 year classes as moderately damaged and those with 0-1 year class as severely damaged. In order to analyse an aquous soil extract 70 g of fresh, well mixed soil were weighed into a 500 ml polyethylene bottle together with 200 ml of twice distilled water and shaken on a Gerhardt model LS 20 shaker for 1 hour. The pH was then measured with a Metrohm model E 488 pH/mV meter and a model EA 152 combined electrode. After centrifugation in a Hereaus Christ 3 labofuge (10 min, 5000 rpm) 100 ml of the supernatant were fixed with 0.5 ml of 200 $mg.l^{-1}$ $HgCl_2$ solution and stored at -28 °C until analysis. For the tissue analyses 50 mg of dried (72 h, 55°C and ground needles were digested according to Nkonge & Ballance (1982).

Nitrification and soil acidification experiments were conducted with a mixture of ten acidic forest soils (pH H_2O 4.1). A portion of the mixture was artificially buffered with 50 mmol $CaCO_3$ kg^{-1} (pH H_2O 6). To 600 g portions of the artificially buffered fresh soil (moisture content 21 %), 0,5, 10, 15, 20, 25, 38 mmol of $(NH_4)_2SO_4$ were added per kg of fresh soil; the untreated mixture received 25 $mmol.kg^{-1}$. All soils were incubated at 20 °C for 114 days, covered with glasswool to prevent evaporation. The pH was measured fortnightlty and the chemical composition was estimated at the beginning and end of the 114 days period.

Nutrient uptake/release experiments were carried out with 1/2 year old needles of a healthy *Pinus nigra* var. *maritima* tree from a forest near Nijmegen. After collection, the needles were washed thoroughly with twice distilled water. Five 50 g (wet weight) portions of needles were incubated (24h, 20 °C) in duplo in 2 l perspex containers, containing 0.5 l bidistilled water and 1: 0 µmol $(NH_4)_2SO_4$, 2: 250 µmol $(NH_4)_2SO_4$, 3: 250 µmol Na_2SO_4, 4: 2500 µmol $(NH_4)_2SO_4$, 5: 2500 µmol Na_2SO_4. The containers were placed on a Gerhardt model 20 shaker and irradiated with a Philips type HP (1) 400 W high pressure metal halide lamp at a light intensity of 500 $\mu E.m^{-2}.s$-1. During the experiment the pH in all media was 4.35 ±0.15. 10 ml water samples were taken at 0, 1, 4, 8 and 24 h after the start of the experiment and analysed immediately after completion of the experiment.

Calcium, magnesium contents were estimated with a Beckman model 1272 A.A.S., and aluminium with a LI type V11 flameless A.A.S. Potassium was estimated flame-photo-metrically using a Technicon I Auto-Analyzer. A Technicon II Auto-Analyzer was used for the colorimetric analyses of nitrate according to Kamphake *et al.* (1967), ammonium by the method of Grasshof and Johannsen (1977), sulphate by the Technicon methodology (1981) and chloride according to O'Brien (1962). Statistical analyses were carried out according to Kruskal-Wallis.

RESULTS

Effects on soil

The ammonium levels of the forest soil and the ammonium sulphate levels in precipitation increase strongly with decreasing distance from chickenfarms (Figure 1). In all forest soils investigated, however, nitrate levels were low and ammonium appeared to be the preponderant nitrogen source.

The average pH (H_2O) was the same for healthy, moderately damaged and severely damaged forests (pH 4.1, Table 1). The results from the incubation experiments demonstrated that nitrification proceeded rapidly in artificially buffered soils leading to a rapid decline in pH (Figure 2). With sufficient ammonium sulphate present the pH decreased to 4.1. The pH did not decrease further in the presence of

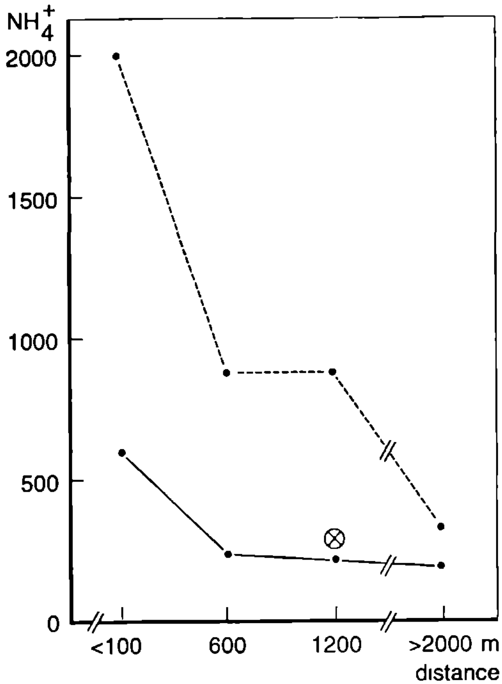

Figure 1: The ammonium concentration in --- open field precipitation (µM) and - - forest soil (µmol.kg^{-1} DW) as a function of the distance to chicken farms.

ammonium sulphate not even after 114 days (Figure 3).

Table 1: pH and chemical composition (averages) of soil distilled water extract (1:3) in A: healthy, B: moderately damaged and C: severely damaged *Pinus nigra* var. *maritima* forests (µmol.kg^{-1} dry soil).

	n	pH (H_2O) Mean	Min	Max	NH_4	NH_4(KCl)	NO_3	K	Mg	Ca	Al
A	20	4.1	3.5	4.6	334	687	271	137	77	153	191
B	16	4.0	3.4	4.9	384	751	130	47	45	128	158
C	20	4.1	3.7	4.4	509	1346	117	60	26	43	183

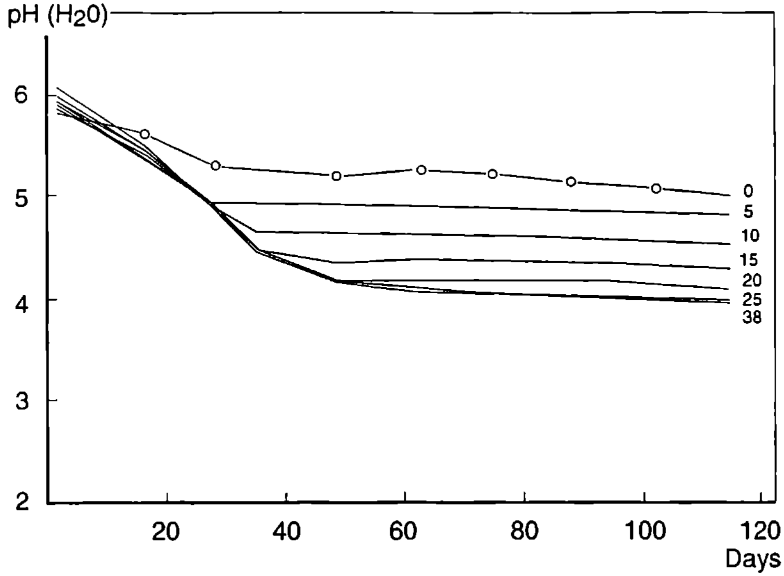

Figure 2: The change in pH of - - acidic and --- artificially buffered heathland soil as a function of the amount of ammonium sulphate added (mmol.kg^{-1} DW).

The NH_4 levels (Table 1) in soil solutions of severely damaged forests are significantly higher than in healthy forests (P=0.045), whereas the Mg and Ca concentrations seem to be lower (P=0.008 and 0.004). Also the K concentrations seem to be lower in the soil of severely damaged forests but these differences were not significant (P=0.08). The aluminium concentrations were almost equal for healthy, moderately damaged and severely damaged forests. As a result the NH_4/K, NH_4/Mg and Al/Ca ratio are relatively low in healthy forests and significantly higher in severely damaged forests (P=0.010, P=0.003 and P=0.017) (Table 2).

Effects on leaves

Typically characteristics of damaged trees are premature shedding of needles and the frequent occurence of fungal diseases. Tissue analyses show that there are only slight differences between the potassium and magnesium levels in one, two and three year old needles of non-damaged trees (Table 3), but the levels in two year old needles of damaged trees appear to be significantly lower (K: P=0.0002, Mg: P= 0.036). The potassium levels and generally the magnesium levels also, are lower than the observed minimum levels in needles of non-damaged trees. The nitrogen levels in one year old needles of severely damaged trees are significantly higher (P=0.0058).

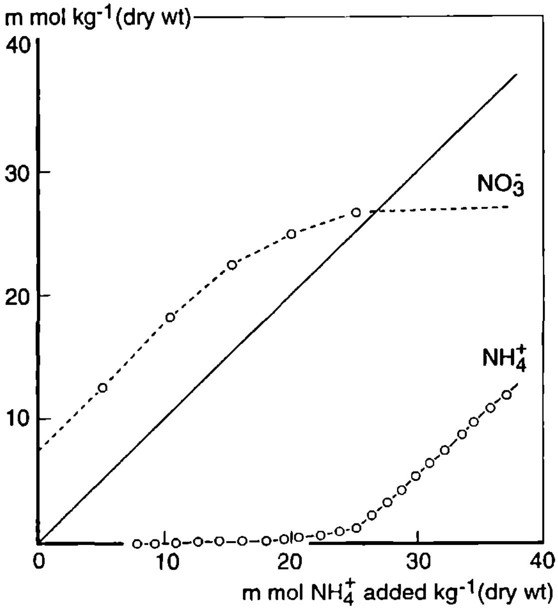

Figure 3. Ammonium and nitrate concentrations in a moderately buffered heathland soil after incubation for 114 days (20 °C) with addition of different amounts of ammonium sulphate.

Table 2: Some nutrient ratios in soil extracts of A: healthy, B: moderately damaged and C: severely damaged *Pinus nigra* var. *maritima* forests (mol/mol).

		NH_4/K			NH_4/Mg			NH_4/Ca			Al/Ca		
	n	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
A	21	4.7	0.5	14.0	6.4	1.1	24.3	4.0	0.3	9.8	2.0	0.4	5.6
B	17	9.2	0.8	36.8	10.0	1.8	26.3	2.9	0.5	9.2	1.3	0.2	2.8
C	21	11.3	1.9	51.8	22.1	1.6	57.2	10.1	0.7	19.8	5.5	1.7	16.7

All trees infected with the fungi *Brunchorstia pinea* (Karst) Höhnel and/or *Diplodea pinea* (Desm.) Kickx had nitrogen levels in the needles exceeding 1150 $\mu mol.g^{-1}$ (DW), whereas for all non-infected trees they were below 1150 $\mu mol.g^{-1}$ (DW) (Table 4).

Table 3: Potassium, magnesium and nitrogen levels in 1, 2 and 3 years old needles of of A: healthy, B: moderately damaged and C: severely damaged *Pinus nigra* var. *maritima* forests (µmol.gr-1 DW).

	A			B			C		
year class	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Potassium									
1	123.8	81.3	150.0	103.2	50.0	128.2	137.0	65.7	325.0
2	112.6	68.8	187.5	43.8	0.0	65.7	Not present		
3	119.4	71.9	215.5	Not present			Not present		
Magnesium									
1	44.1	17.6	80.0	30.1	12.5	51.3	34.1	10.6	55.0
2	33.5	20.0	45.0	22.7	13.1	42.5	Not present		
3	31.8	21.3	56.3	Not present			Not present		
Nitrogen									
1	944	800	1117	1006	844	1478	1269	883	1517
2	878	656	1094	969	544	1561	Not present		
3	828	628	1044	Not present			Not present		

Table 4: Nitrogen levels in one year old needles of trees infected by *Brunchorstia pinea* (Karst) Höhnel and/or *Diplodea pinea* (Desm.) Kickx and in non-infected trees (µmol.gr_{-1} DW).

Infected (n=11)			Non-infected (n=17)		
Mean	Min	Max	Mean	Min	Max
1355	1189	1517	956	800	1117

Analyses of canopy throughfall and open field precipitation show clear differences in chemical composition (Table 5). In open field precipitation sulphate is fully compensated by ammonium, but only ±80% in canopy throughfall, the remainder by potassium, magnesium and calcium. Ammonium sulphate levels in canopy throughfall can increase to 4000 µmol.l^{-1} in areas with high ammonia emission.

The ecophysiological experiments show extensive ammonium uptake by needles and K, Mg and Ca release, when they are incubated in an ammonium containing medium (Figure 4). This process proceeds continuously. When the needles are added to a medium with a certain salt concentration, rapid excretion of K, Mg, Ca takes place initially, irrespective of whether $(NH_4)_2SO_4$ or Na_2SO_4 has been used. In a medium containing Na_2SO_4 however, the excretion of cations decreases

Table 5: The average chemical composition of precipitation in a: open plots (2) and b: throughfall (4) in a *Pinus nigra* forest in an area with intensive stockbreeding ($\mu mol.l^{-1}$)

	NH_4	NO_3	K	Mg	Ca	SO_4	Na	Cl	H
a	597	71	53	35	71	318	170	197	1.7
b	2283	147	196	94	150	1379	346	462	0.6

rapidly after a few hours and gradually becomes comparable with that of needles in twice distilled water, whereas in a medium containing $(NH_4)_2SO_4$ the needles continue to excrete relatively large amounts of K, Mg, Ca (Figure 5, 6 and 7). The duplo experiments varied within a few percent. In media with relatively low $(NH_4)_2SO_4$ levels, the excretion of Ca and Mg is more than ten times higher than in distilled water.

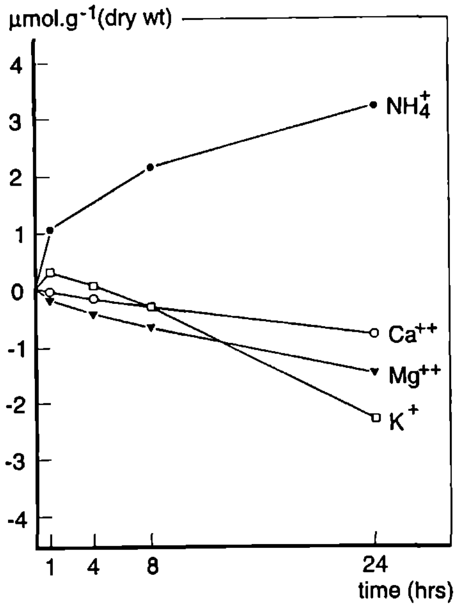

Figure 4. Ammonium uptake and potassium, magnesium and calcium release from pine needles in a 250 μM ammonium solution.

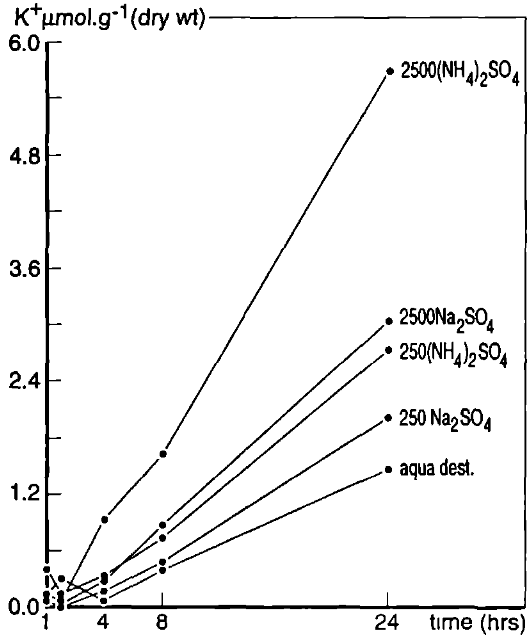

Figure 5: Potassium release from pine needles in distilled water and in in solutions containing ammonium and sodium sulphate (µM).

DISCUSSION

A clear relation exists between the distance from stock farms and the ammonium sulphate deposition (Roelofs *et al.*, 1984a). The deposited ammonium sulphate affects the forest ecosystem. The nutrient release/uptake experiments show that needles of *Pinus nigra* var. *maritima* take up ammonium from $(NH_4)_2SO_4$ solutions and compensate by excreting potassium, magnesium and calcium, which cannot be ascribed exclusively to an osmoregulatory adaptation. This phenomenon has also been described for aquatic macrophytes in acidified $(NH_4)_2SO_4$ containing waters (Roelofs *et al.*, 1984b). Forest canopy throughfall analyses suggests a further confirmation of this exchange mechanism. Tissue analyses demonstrate that the observed premature shedding of the needles of damaged trees is related to potassium and/or magnesium deficiencies in older needles. Krause *et al.* (1984) also indicate nutrient deficiencies as a result of cation leaching in German forests, but they describe this mainly to needle damage caused by ozone. However, this cation leaching is rather

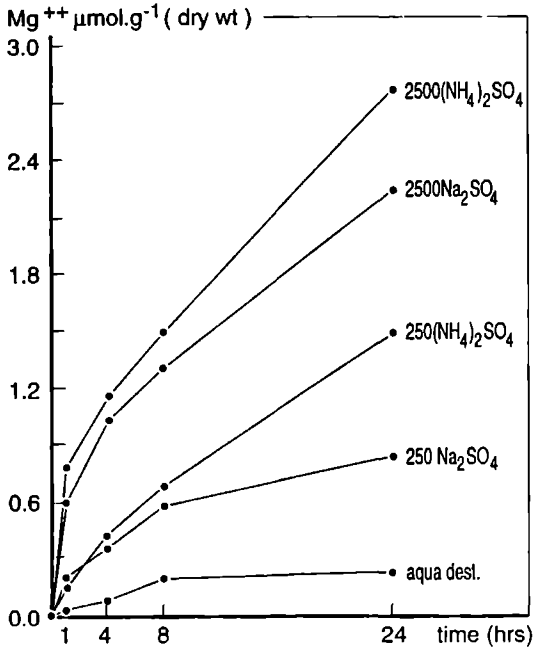

Figure 6: Magnesium release from pine needles in distilled water and in solutions containing ammonium and sodium sulphate (µM).

low compared with that caused by ammonium sulphate deposition.

The above mentioned reactions help to explain the high nitrogen levels in pine needles.

All trees investigated and infected by *Brunchorstia pinea* (Karst) Höhnel and *Diplodea pinea* (Desm.) Kickx showed higher total N in the needles. A possible causal relation between the high N Levels and susceptibility to fungal diseases has to be further investigated.

Several authors (Van Breemen *et al.*, 1982; Van Breemen & Jordens, 1983; Klein *et al.*, 1983; Kriebitzsch, 1978) mention nitrification of ammonium and concomittant soil acidification in acidic forest soils. In this study the acidic forest soils investigated seem to show little or no nitrification; the nitrate levels were relatively low and ammonium levels were high. The incubation experiments with artificially buffered soils demonstrated that in these soils nitrification stops or is strongly inhibited at pH (H_2O) 4.1. Soils of healthy, moderately damaged and severely damaged forests had an average pH (H_2O) of 4.1, indicating that the soil pH probably

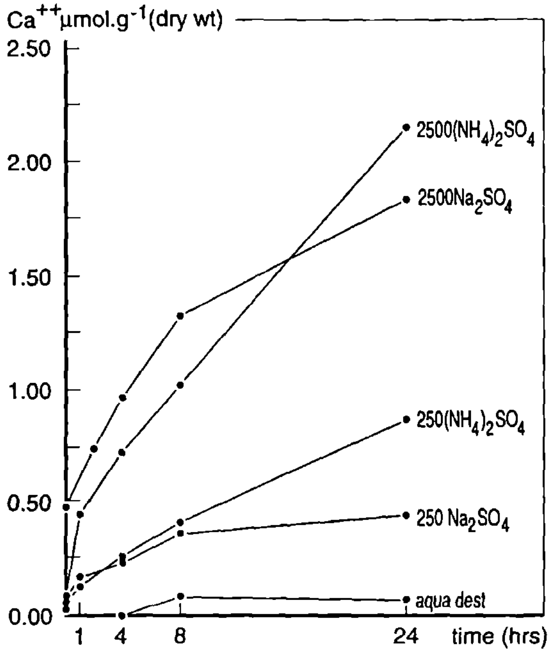

Figure 7: Calcium release from pine needles in distilled water and in solutions containing ammonium and sodium sulphate (µM).

is determined by the nitrification limit. It should be noted that all the *Pinus nigra* forests investigated han been planted on former acidic heathland soils, so the nitrification results are consistent with those of Kriebitzsch (1978), who conducted nitrification experiments in many types of acidic forest soil.

The observed ammonium sulphate deposition also results in cation exchange and consequent cation leaching (K, Mg and Ca) from the soil. The NH_4/K, NH_4/Mg and NH_4/Ca ratios are much higher in soils of severely damaged trees. It has already been stated by other authors (Mulder 1956; Jacob, 1958) that these increased ratios, especially in the soils with a low nutrient level, inhibit nutrient uptake.

The aluminium levels in soils of non-damaged forests are almost equal to those in moderately and severly damaged forests. However, as a result of calcium leaching, the Al/Ca ratio is much higher in soils of severely damaged forests.

Ulrich (1983) mention a Al/Ca ratio > 5 as very critical for root damage. In soils of severely damaged forests this ratio was indeed above 5, but some healthy forest also showed Al/Ca ratios far above this limit. However according to Ulrich

(1983) a high Al/Ca ratio does not necessarily results in damage to trees. Organic acids in the soil can detoxify aluminium by forming organic aluminium complexes.

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CHAPTER 3

DEPOSITION OF ACIDIFYING AND EUTROPHICATING SUBSTANCES IN DUTCH FORESTS

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ABSTRACT

In order to qualify and quantify the acidifying and eutrophicating substances that contribute to atmospheric deposition in Dutch coniferous forests open field precipitation and throughfall water have been sampled monthly at 14 locations in The Netherlands over an 18-month period. Spatial variation was studied by comparing the chemical composition of deposition in forests of four regions. Within the forests, throughfall fluxes of different tree species were compared. In this paper the deposition fluxes of sulphuric and nitrogenous compounds are emphasized.

This study proves that most forested areas in The Netherlands receive high loads of nitrogenous (5.6 kmol $N.ha^{-1}.yr^{-1}$) and potentially acidifying (9.4 kmol $H^{+}.ha^{-1}.yr^{-1}$) compounds. Particularly, deposition of ammonium and sulphate is high, not only in areas with intensive agricultural activities, but throughout the country. Regional differences in deposition appear to be relatively small. With the exception of the two coastal locations, deposition shows little spatial variation. In the coastal region loads of nitrogenous and potentially acidifying compounds are lower than elsewhere in the country. Generally higher throughfall fluxes have been measured in Douglas fir stands than in pine stands. The larger surface area of firs probably accounts for an enhanced dry deposition. In the summer season throughfall fluxes are generally smaller than in the winter season.

INTRODUCTION

The enormous increase of pollutants in the atmosphere during the last decades has caused a dramatic change in the chemical composition of atmospheric deposition. Ozone, nitrogen oxides and sulphate dioxide are accepted to be the pollutants affecting the environment on a global scale.

In The Netherlands the first observations of forest damage were made in the south-eastern part of the country. The regional character of this forest decline indicated a regional cause. None of the above mentioned pollutants did fit the damage pattern. However, ammonia emission and ammonium deposition appeared to be extremely high in the affected area. Recent research has indicated that a high atmospheric ammonium input leads to acidification and eutrophication of the forest soil and consequently adversely affects the vegetation (Van Breemen *et al.*, 1982; Roelofs *et al.*, 1985).

In the Netherlands 91 % of ammonia present in the atmosphere is directly emitted from livestock farms or evaporates from liquid-manured, arable land (Ivens, 1990). While most ammonia emitting sources are situated near ground level, atmospheric transport of this compound was always supposed to be small compared to that of other pollutants. Consequently deposition of both ammonia and ammonium was expected to take place at a relatively short distance from the source. The local character of the initial forest damage confirmed these assumptions. Asman (1987) found that gaseous ammonia is indeed deposited close to the source but that ammonium sulphate aerosoles can be transported over long distances.

On wet plant surfaces or in humid air ammonia reacts with sulphate dioxide to form ammonium sulphate. Consequently large amounts of ammonium and sulphate are filtered from the atmosphere by the tree canopy (van Breemen *et al.*, 1982; Grennfelt & Hultberg, 1986; Draaijers *et al.*, 1988). Apart from ammonium and sulphate, fluxes of various other compounds are altered due to down-wash of particles or gases deposited on the plant surface and exchange of substances by the tree canopy (Lindberg *et al.*, 1986; Freiesleben *et al.*, 1986). After the passage of rainwater through the canopy the actual load of pollutants to the forest floor can be estimated. Leaving aside canopy exchange, throughfall measurements give good estimates of atmospheric deposition as stemflow contributes at most 8 % to the total load on forest soils and, therefore, can be neglected. (Van Breemen *et al.*, 1982; Miller, 1984; Bredemeier, 1988).

Investigations of the Dutch State Forest Service have shown that forest deterioration is no longer restricted to areas with dense concentrations of agricultural activities but also has expanded to other parts of the country (Anonymous, 1984; Anonymous, 1990). The major aim of the present research was to find out whether forest damage in those areas could also be caused by an enhanced ammonium deposition. Therefore, qualification as well as quantification of atmospheric input to Dutch forests was essential. To investigate which acidifying and eutrophicating substances contribute to atmospheric deposition in forests, throughfall collectors were used. In order to establish regional differences in throughfall deposition, collectors were placed at 14 locations all over the country. Variation in throughfall fluxes within a forest was studied by comparing throughfall deposition under three coniferous tree species. The seasonal variability of atmospheric deposition will also be discussed.

MATERIALS AND METHODS

In November 1986, 14 forest locations in The Netherlands were selected. All forest stands consisted of coniferous trees planted on weakly buffered, sandy soils. The investigation concerned Corsican pine (*Pinus nigra* var. *maritima* (Ait.) Melville), Scots pine (*Pinus sylvestris* L.) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). The selection of the sampling sites was primarily based on the occurrence of all three coniferous tree species within a distance of 5 km of each other. At each location one representative specimen of each species was chosen for the collection of throughfall water. The influence of local ammonia sources such as livestock farms and liquid-manured arable land was restricted by selecting relatively large forests and avoiding the edges of the forest. Furthermore, 25-40-year-old stands were chosen to diminish variations caused by age differences. However, neither tree vitality nor the occurrence of illnesses or infections were considered when selecting the sites.

After selection of the stands the locations were grouped into four regions according to their geographical situation; Coast, North, Centre and South. Figure 1 shows the distribution of the sampling locations.

Figure 1:Geographical position of the sampling locations and their regional division in The Netherlands. Coastal region: 1=Terschelling, 2=Schoorl; Northern region: 3=Bakkeveen, 4=Diever, 5=Orvelte, 6=Hardenberg; Central region: 7=Lunteren, 8=Loenen, 9=Heumen; Southern region: 10=Hulst, 11=Ossendrecht, 12=Breda, 13=Maarheeze, 14=Meyel

Deposition collectors were placed in October 1986. For 18 months, open field precipitation (bulk) as well as throughfall water were collected. At each location one rain sampler was placed in a clearing near the forest stand and two samplers were placed within the forest stand at 0.75 m distance from the stem of the selected tree, for this purpose.

The collectors consisted of a black, 2 l polyethylene bottle and a polyethylene gauge with an aperture of 74.5 cm^2. On the bottom of this gauge a plastic filter was inserted in order to avoid organic matter and/or insects contaminating the water in the bottle. Bottle and gauge were placed in a plastic pipe dug in the ground. The opening of the gauge was 20 cm above ground level. In each collecting bottle, 2 ml of a solution containing 200 $mg.l^{-1}$ mercury chloride was added to inhibit microbial activity. After each month, the samples were collected and the bottles and gauges were cleaned with demineralized water. The volume was measured in the field, pH was determined at the laboratory within 24 h after collection. After that, samples were stored at -28 °C until further analysis.

A Radiometer type PHM 82 standard pH-meter and a combined electrode was used to measure pH. Calcium, magnesium, aluminum, phosphorus and sulphur were measured with an Inductively Coupled Plasma spectrophotometer (ICP), type IL Plasma 200. To check that the ICP could be used to estimate the sulphate content of the rainwater a colorimetrical method using a Technicon AAI-system (Anonymous, 1969) was employed. Potassium and sodium were estimated using a Technicon Flame photometer IV. Colorimetrical determination with a Technicon AAII-system according to Technicon Methodology (Anonymous, 1969) and Kempers & Zweers (1986) was used for nitrate and ammonium respectively, and a Technicon AAI-system according to Technicon Methodology (Anonymous, 1969) and O'Brien (1962) for chloride.

Acidifying sulphuric and nitrogenous compounds amount to the total load of potentially acidifying compounds (potential acid) in throughfall water. As part of these acidifying compounds are counteracted by the deposition of alkaline substances, the total flux of potential acid can be calculated as the summed volume weighted deposition of $2xSO_4 + NH_4 + NO_3 - 2xCa$ in $kmol.ha^{-1}.yr^{-1}$. (Buysman, 1990).

Leaving aside canopy interaction, throughfall deposition is equivalent to total atmospheric deposition in forests. Total deposition can be subdivided into wet and dry deposition (Ivens, 1990). According to Bredemeier (1988) for some constituents the canopy may act like an inert sampler, all dry deposited material is washed off the surfaces during rain events and appears in throughfall water. This can be assumed for elements that are deposited at very high rates compared to their internal cycle (e.g. sulphate in Central Europe). Ivens (1990) suggests that this probably also applies for the nitrogen deposition in The Netherlands. Based on these assumptions dry deposition of sulphuric and nitrogenous compounds can be calculated as the difference between throughfall and bulk deposition.

Annual deposition fluxes were calculated as the sum of all measured monthly concentrations multiplied by the monthly volume of precipitation. Seasonal fluxes were studied by comparing two winter seasons (November- 1986-April 1987 and November 1987 - April 1988) with one summer season (May 1987 - October 1987). Throughfall deposition per tree was calculated as the mean flux of two similar collectors. From annual or seasonal fluxes at the sampling sites 50 percentile (median) values were calculated. Prior to statistical analysis values of these fluxes were log-transformed to make the variance independent of the mean (Sokal & Rohlf, 1981). Scheffé's multiple-comparison procedure was performed to establish the influence of regional and temporal variability on various components of the deposition. These statistical analyses were performed with the General Linear Models (GLM) procedure available in the Statistical Analysis System (SAS) software package (Anonymous, 1985).

RESULTS

Regional differences

Between November 1986 and November 1987 the amount of precipitation measured in The Netherlands by using bulk collectors was 1060 mm (Table 1). In forests, 669 mm of throughfall water was collected, indicating that 33 % was lost due to interception by the canopy. In the coastal region the amount of precipitation was lowest in both bulk and throughfall samplers. However, the difference between this and the other regions was not significant. The variation in the amounts of precipitation in stands of different species within the same area was often larger than the variation between the regions.

Table 1: Median values (50% percentile) of the annual amount of precipitation (mm) collected in bulk and throughfall samplers between November 1986 and November 1987 on a regional and national scale based on 14 locations in The Netherlands. None of the differences within the columns are significant according to Scheffé's multiple-comparison procedure.

	bulk precipitation	Corsican pine	Scots pine	Douglas fir	median throughfall
Coastal region	812	627	539	-	608
Northern region	1067	779	695	674	728
Central region	1125	710	753	550	715
Southern region	1044	511	719	669	663
National	1060	626	719	652	669

In the coastal region the deposition of potential acid was lowest, both in bulk and in throughfall. Significant differences in bulk and throughfall could only be established between the coastal region and the central and southern regions (Table 2). In all investigated forests about 75 % of potential acid (± 7.2 kmol $H.ha^{-1}.yr^{-1}$) was deposited in a dry form.

Table 2: Median fluxes of potential acid and total nitrogen in bulk and throughfall collectors and the amount of dry deposited potential acid in $mol.ha^{-1}.yr^{-1}$ between November 1986 and November 1987 on a regional and national scale based on 14 locations in The Netherlands. Different letters within each column indicate statistical difference at the 5% level according to Scheffé's multiple-comparison procedure.

	potential acid			total nitrogen	
	bulk	throughfall	dry deposition	bulk	throughfall
Coastal region	1516a	5382a	3458a	925a	3548a
Northern region	2151ab	9990ab	7729ab	1415b	5978ab
Central region	2748b	10130b	7644ab	1480b	5952ab
Southern region	2416b	11222b	8842b	1325b	6544b
National	2154	9453	7187	1366	5556

In the coastal region the contribution of sulphate and nitrate to the amount of total potential acid in throughfall deposition was highest, 55% and 16% respectively (Table 3). In this region significantly higher sulphate and nitrate and lower ammonium deposition was measured. On an average sulphate contributed 46%, ammonium 44% and nitrate 10% to the load of potential acid in Dutch forests.

In all regions in throughfall as well as in bulk deposition annual mean $(NH_4)_2SO_4$ molar ratios were 1.0 (data not shown).

In the coastal region the nitrogen flux in bulk deposition was significantly lower than that in the deposition in the other regions (Table 2). In throughfall, nitrogen deposition was only significantly lower in the coastal area compared to the southern region. However, the amount of ammonium deposited at the coast was significantly different from all other regions in bulk as well as in throughfall. In the stands studied the total nitrogen deposition amounted to ± 5.6 $kmol.ha^{-1}.yr^{-1}$. (=78 kg $N.ha^{-1}.yr^{-1}$) of which 81% was deposited as ammonium (data not shown).

Table 3: Median values of the contribution of acidifying sulphuric and nitrogenous compounds to the total potential acid flux in bulk and throughfall collectors (in percentages) between November 1986 and November 1987 on a regional and national scale based on 14 locations in The Netherlands. For the explanation of significance symbols see Table 2.

	bulk			throughfall		
	SO_4	NH_4	NO_3	SO_4	NH_4	NO_3
Coastal region	48a	30a	22a	55a	29a	16a
Northern region	44a	38ab	18a	43c	47b	10b
Central region	43a	40b	17a	45bc	45b	10b
Southern region	41a	41b	18a	49b	42b	9b
National	43	39	18	46	44	10

Differences between tree species

The median annual amounts of nitrogen and potential acid deposited under each tree species per region are shown in Table 4. On a regional scale none of the differences between fluxes per tree species were significant. The median loads of nitrogen and potential acid were highest in the Douglas fir stands (6.7 kmol $N.ha^{-1}.yr^{1}$ and 11.3 kmol $H.ha^{-1}.yr^{1}$ respectively). The median nitrogen fluxes in Corsican and Scots pine stands were almost similar and amounted to 4.9 and 4.8 kmol $N.ha^{-1}.yr^{1}$ respectively. The deposition of potential acid in Scots pine stands was lower (8.3 kmol) than the deposition in Corsican pine stands (8.6 kmol $H.ha^{-1}.yr^{1}$). However, significant differences existed only in nitrogen deposition between Douglas fir and Scots pine (Table 4).

Table 4: Median fluxes of total nitrogen and potential acid in throughfall collectors of Corsican pine (cp), Scots pine (sp) and Douglas fir (df) in $kmol.ha^{-1}.yr^{-1}$. between November 1986 and November 1987 on a regional and national scale based on 14 locations in The Netherlands. Different letters within a line indicate statistical difference at the 5% level according to Scheffé's multiple-comparison procedure.

	total nitrogen			potential acid		
	cp	sp	df	cp	sp	df
Coastal region	3.9	3.1	-	5.6	4.8	-
Northern region	5.8	5.2	6.2	9.9	8.9	10.0
Central region	5.1	4.6	9.3	8.8	7.9	14.7
Southern region	4.7	6.0	7.1	7.9	10.4	12.0
National	4.9ab	4.8a	6.7b	8.6a	8.3a	11.3a

Seasonal differences

In the summer season potential acid in bulk deposition was significantly higher than in the winter seasons (Table 5). However, in throughfall significantly lower fluxes of potential acid were measured in the summer season. In bulk deposition the amounts of ammonium, sulphate and nitrate were significantly higher in summer. In this season throughfall contained a significantly higher amount of nitrate and a lower amount of sulphate. Potassium, magnesium and calcium showed also seasonal variation in throughfall. Depositions of sodium and chloride were significantly lower in summer than in winter in bulk and in throughfall.

Table 5: Median fluxes per season in bulk and throughfall collectors in $mol.ha^{-1}.0.5yr$-1 on a national scale based on 14 locations in The Netherlands. w86=winter 1986, s87=summer 1987 and w87=winter 1987. Different letters within a row and per sample type indicate statistical difference at the 5% level according to Scheffé's multiple-comparison procedure.

	bulk			throughfall		
	w86	s87	w87	w86	s87	w87
pot. acid	874a	1290b	890a	5470a	3728b	5013ab
NH4	418a	571b	420a	2652a	2027a	2755a
NO3	151a	284b	172c	358a	596b	379a
SO4	237a	278b	184a	1541a	816b	1222a
Mg	49a	48a	50a	183a	113b	156a
Ca	72ab	77a	50b	242a	163b	207ab
K	69a	123a	104a	342a	512b	315a
Na	440a	215b	520a	1293a	589b	1084a
H	225a	195a	117a	153a	50b	46b
Cl	457a	309a	803b	1183a	676b	1432a

The relative contribution of nitrogen oxides to potential acid in throughfall deposition was significantly higher in the summer season (Table 6). This increase was accompanied by a significant decrease of the relative contribution of sulphate. The contribution of ammonium was not significantly affected by the change of season. The relative contributions of ammonium and sulphate showed similar seasonal trends in bulk and throughfall deposition.

Table 6: Median values of the contribution of acidifying sulphuric and nitrogenous compounds to the total potential acid flux in bulk and throughfall collectors (in percentages) per season on a national scale based on 14 locations in The Netherlands. w86=winter 1986, s87=summer 1987 and w87=winter 1987. For the explanation of significance symbols see Table 2.

	bulk			throughfall		
	SO_4	NH_4	NO_3	SO_4	NH_4	NO_3
w86	47a	38a	15a	51a	42a	7a
s87	40b	41a	19a	40b	45a	15b
w87	46a	39a	15a	47c	46a	7a

DISCUSSION

Regional differences in deposition appeared to be small. Actually, only the composition of the deposition in the coastal area differed from that of other parts of the country. In this region, the lowest amounts of potential acid and of total nitrogen were measured. This lower load was primarily due to the lower ammonium fluxes. The prevailing south-westerly wind mainly transports air from the sea which contains hardly any NH_x. At all other sites, provided they are not too close to sources, background emission density probably determines deposition (Schuurkes *et al.*, 1986). In the forests at the coast, total nitrogen deposition amounted to 50 kg $N.ha^{-1}.yr^{-1}$ of which 64% was due to the deposition of ammonium. However, in all investigated Dutch forests, nitrogen deposition amounted to 80 kg $N.ha^{-1}.yr^{-1}$ of which 84% was deposited as ammonium. Similar throughfall fluxes of ammonium have been measured by other investigators in The Netherlands (Kleyn *et al.*, 1988; van Breemen & van Dijk, 1988; Ivens, 1990). Throughfall measurements in Scots pine stands in the UK revealed a much lower nitrogen deposition of 15 kg to 18 kg $N.ha^{-1}.yr^{-1}$ (Alcock & Morton, 1985; Skeffington, 1983). Nilsson (1987) reported N-deposition of 30 to 40 kg ha.-1.yr-1 with peak values of above 60 kg ha.-1.yr-1 in Central Europe.

Duyzer *et al.* (1988) calculated deposition fluxes by estimating deposition rates and using emission data. They estimated the NH_x contribution to the load of nitrogen and potential acid at 60 % and 30 %, respectively. Only in the coastal area those estimated NH_x contributions equalled the measured contributions. In all other regions the measured contributions were higher, 84% and 44% respectively. These discrepancies can be explained by the fact that in calculations by Duyzer *et al.* (1988) co-deposition of ammonium and sulphate is not taken into account. Particularly in polluted areas concentrations of ammonium and sulphate mutually enhance their deposition rates (Adema, 1986).

According to Bücking & Steinle (1987) forest structure characteristics such as species distribution and crown cover account for spatial distribution of deposition within a forest. Catching of aerosols and small dry particles is influenced by the shape and size of the catching surface. Turbulence and deposition rate are small on a homogeneous surface and deposition is large in closed stands (Bücking & Steinle, 1987). Draaijers *et al.* (1988) ascribe higher throughfall fluxes in Douglas fir stands compared to those in deciduous stands to the relatively larger foliage surface. In this study the local variation was probably also primarily due to differences in dry deposition as a result of variation in the size of the catchment surface between pine and fir stands.

On a local scale variations in deposition are often larger than the variation between the regions. The amount of nitrogen and potential acid in throughfall deposition appeared to be more dependent on the individual characteristics of the stands at a location than on the geographical situation of the region. At places where the stands border an area with less high vegetation (forest edge) throughfall fluxes are enhanced (Ivens, 1990). Investigations of Ivens (1990) showed that in stands near emission sources enhanced throughfall fluxes can be measured at 100 m from the forest edge. According to Hasselrot & Grennfelt (1987) deposition fluxes are already enhanced at the transition of a stand with smaller to one with larger trees. This phenomenon probably also contributes to the local variation in deposition fluxes within one region, as forest structure characteristics like forest size and species composition differ widely between the locations.

Apart from affecting the amount of deposition due to height differences tree age can also affect the chemical composition of throughfall deposition. Canopy exchange processes can strongly vary with tree age. In areas with a high input of actual acid, more acid is measured in throughfall deposition of older stands (Miller, 1984). N-demands of older stands appear to be smaller than those of young stands (Wilson & Pitcairn, 1988). In areas with low N deposition, amounts of ammonium and nitrate in throughfall deposition may even be smaller than those in bulk deposition (Lovett & Lindberg, 1984; Persson & Broberg, 1985; Grennfelt & Hultberg, 1986).

Bulk and throughfall displayed opposite seasonal trends for sulphate and ammonium fluxes (table 5). The enhanced throughfall fluxes during winter are related to high emissions in this season and to the increased co-deposition under wet conditions. Bulk fluxes of ammonium and sulphate are also expected to be higher in winter. The enhanced bulk fluxes in the summer season of this study are probably due to the high amount of precipitation in the summer of 1987. These findings are in agreement with measurements of the Netherlands Precipitation Chemistry Network. In that research increased amounts of precipitation and correspondingly enhanced fluxes of ammonium and sulphate were measured in summer 1987 compared to the summer seasons in 1986 and 1988 (Anonymous, 1987; Anonymous, 1988). In bulk as well as in throughfall, nitrate deposition is higher in summer than in winter. This difference can be caused by higher emission of nitrogen oxides but also by partial nitrification of dry deposited ammonium. Potassium, magnesium and calcium only varied in throughfall deposition. The seasonal difference for these cations is probably caused by canopy exchange in summer. During dormancy in winter, this process is supposed to be negligible.

In conclusion, this study has proved that most forested areas in The Netherlands receive high fluxes of pollutants, in particular those of ammonium and sulphate, and that this problem is not restricted to areas with intensive agricultural activities. In fact only the coastal area receives lower loads of nitrogen and potential acid. However, even in this region, the critical loads for acid (1600 eq) (Schneider & Bresser, 1988) and nitrogen (1400 eq) (Boxman *et al.*, 1988) are severely exceeded. Throughfall fluxes of nitrogen and potential acid varied with tree species. Species composition of the forest seems to cause more spatial variation in the deposition than the geographical position of the forested area. The relative contributions of ammonium and nitrate to total nitrogen as well as those of ammonium and sulphate to potential acid varied little. The equal contributions of ammonium and sulphate indicate co-deposition of those compounds. Seasonal and annual variations in deposition indicate that long-term monitoring of throughfall fluxes remains necessary for the estimation of the load of pollutants to the forest soils.

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CHAPTER 4

THE EFFECTS OF ATMOSPHERIC NITROGEN DEPOSITION ON THE SOIL CHEMISTRY OF CONIFEROUS FORESTS IN THE NETHERLANDS

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ABSTRACT

Nitrogen fluxes, particularly those of ammonium are extremely high in Dutch forests. In soils exposed to high ammonium deposition, acidification, eutrophication or a combination of both processes may occur. In addition to the amounts of ammonium deposited, the rate of soil nitrification determines which process takes place.

A nation-wide investigation, in which three coniferous tree species were involved, was carried out to study the relation between deposition fluxes, measured by means of throughfall and bulk samplers, and the chemical composition of the soil.

The ammonium deposition accounted directly for the high ammonium content and the high ammonium/cation ratios in the soil. In the top layer of most of the forest soils which were investigated nitrification rates were low. In these stands ammonium/cation ratios in the soil often reflected ammonium/cation ratios in throughfall water. Even in soils with relatively high nitrification rates, ammonium concentrations exceeded those of nitrate in the top layer of the mineral soil, indicating that ammonium deposition was more important than nitrification rate in determining the predominant form of nitrogen.

INTRODUCTION

Most coniferous forests in the Netherlands were planted in the beginning of this century on nutrient-poor, sandy soils. As a result of the low nutrient status and low buffering capacity, these soils are very susceptible to changes in the mineral balance. Excessive nitrogen deposition may be a major cause of the observed changes in the chemical composition of the soil and consequently of forest decline.

Deposition fluxes in forests are generally larger than those in the surrounding meadows. The increase in concentrations of compounds in rain water when this passes through the tree canopies is particularly large for ammonium and sulphate (Van Breemen *et al.*, 1982; Houdijk & Roelofs, 1991). In soils exposed to high ammonium deposition acidification, eutrophication (increased nitrogen availability) or a combination of both processes may occur. In Figure 1 these processes are schematically described. The soil type strongly determines which process takes place (Van Breemen *et al.*, 1982; Roelofs, 1986). Severe acidification is bound to take place in soils with a relatively high rate of nitrification (oxidation of ammonium to nitrate and the concomitant release of protons). The increasing soil acidity induces loss of exchangeable base cations (K, Mg and Ca) and the release of soluble aluminium (Ulrich, 1983; Van Breemen *et al.*, 1983) resulting in high aluminium/calcium ratios in the soil solution. In soils with no or relatively low nitrification rates enhanced ammonium fluxes may lead to accumulation of ammonium in the top layer of the mineral soil and the loss of potassium, magnesium and calcium to deeper soil layers. This results in increased ammonium/cation ratios (Roelofs *et al.*, 1985). In both soil types increased nitrogen deposition may directly or indirectly account for the mineral imbalance of the soil.

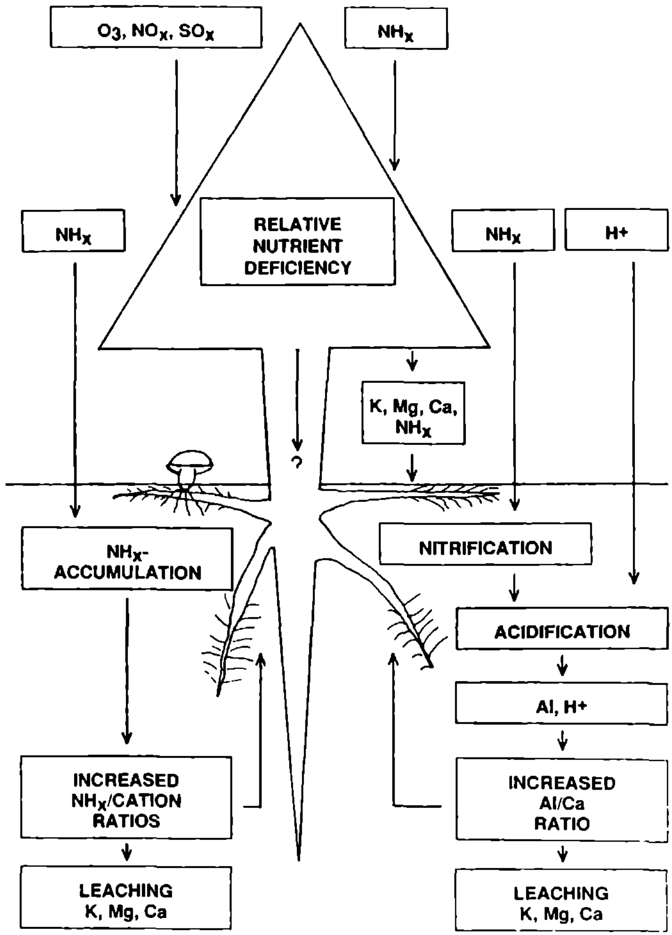

Figure 1: Schematic presentation of processes involved in forest decline.

Field investigations in the southern part of the Netherlands clearly demonstrated a correlation between the mineral balance in the forest soils and the condition of the trees (Roelofs *et al.*, 1985). High ammonium/potassium and ammonium/magnesium ratios were often measured in soils of less vital forests, whereas healthy forests were generally found on soils with lower ratios.

This study was carried out to investigate whether the present, high deposition fluxes in forests are directly responsible for the disturbance of the mineral balance in the forest soil. In this paper the results of a nation-wide research are presented. Soil samples were collected at regular intervals during one year in coniferous forests at 14 locations. At each sampling site both the mineral balance in the soil and the relative nitrification rate were estimated. Besides soil samples, throughfall and bulk water was also collected to establish the impact of deposition fluxes on the mineral balance. The variation in deposition fluxes of acid and acidifying substances in Dutch forests and the impact of these fluxes, directly or indirectly via the soil, on the trees are discussed by Houdijk & Roelofs (1991, 1993).

MATERIALS AND METHODS

In November 1986, 36 forested sites at 14 locations in The Netherlands were selected. All forest stands consisted of coniferous trees planted on weakly buffered, sandy soils. The investigation concerned 13 Corsican pine (*Pinus nigra* var. *maritima* (Ait.) Melville), 13 Scots pine (*Pinus sylvestris* L.) and 10 Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands. The selection of the sampling sites was primarily based on the occurrence of all three coniferous tree species within a distance of 5 km from each other. Furthermore, 25-40-year-old stands were chosen to diminish variations caused by age differences. Neither tree vitality nor the occurrence of illnesses or infection were considered when selecting the stands. After selection, the locations were divided into four regions according to their geographical position: Coast, North, Centre and South (Figure 2). In each stand one representative tree was selected. At that site soil and throughfall samples were collected regularly. At each location a non-forested site was chosen to sample bulk deposition. Soil samples were collected also at this site. More detailed information about the sampling sites and methods can be found in a previous paper describing the atmospheric deposition on the locations (Houdijk & Roelofs, 1991).

During one year, soil samples were collected every two months at each of the 36 forested and 14 non-forested sites. At each site, four subsamples were taken from the mineral soil (0- 15 cm) at 1 m distance from the stem of the tree or, at the open sites, from the bulk collector and mixed. Extractions with bidistilled water or 0.2 M NaCl were made in 500 ml pothylene bottles of 70 g fresh soil with 200 ml extractant. The bottles were shaken for 1 h, and pH was measured directly. After centrifugation for 15 min at 275,000 g, the supernatant was stored at -28°C until analysis.

In both soil extracts Ca, Mg, Al, Fe, Mn, Zn, S and P were measured with an Inductively Coupled Plasma spectrophotometer (ICP), type IL Plasma 200. To check

Figure 2:Geographical position of the sampling locations and their regional division in The Netherlands. Coastal region: 1=Terschelling, 2=Schoorl; Northern region: 3=Bakkeveen, 4=Diever, 5=Orvelte, 6=Hardenberg; Central region: 7=Lunteren, 8=Loenen, 9=Heumen; Southern region: 10=Hulst, 11=Ossendrecht, 12=Breda, 13=Maarheeze, 14=Meyel

that the ICP could be used to estimate the sulphate content of the water extracts a colorimetrical method using a technicon AAI-system (Technicon Corporation, 1969) was employed. Potassium and Na were assessed using a Technicon Flame photometer IV. Colorimetrical determination with a Technicon AAII-system according to Technicon Methodology (Technicon Corporation, 1969) and Kempers & Zweers (1986) was used for NO_3 and NH_4 respectively, and a Technicon AAI-system according to Technicon Methodology (Technicon Corporation, 1969) and O'Brien (1962) for Cl.

The nitrification rates in the soil of the forested sites were estimated indirectly. According to Van Breemen & Van Dijk (1988) chloride can be used as an indicator of the degree of evaporative concentration of meteoric water. Comparison of NO_3/Cl ratio in throughfall deposition and soil solution can be used to determine whether high NO_3 concentrations are due to evapotranspiration, or whether nitrification is involved too.

Scheffé's multiple-comparison procedure (performed with the General Linear Models (GLM) procedure) was used to establish the impact of regional variability on the nutrional status of the soil. The Pearson-test was applied to determine the relation between deposition fluxes (annual total) and the chemical composition of the soil (annual mean) (SAS Institute Inc., 1985). Prior to statistical analysis the data were log-transformed to make the variance independent of the mean (Sokal & Rohlf, 1981). For presentation the data were backtransformed. Consequently, the mean data given are geometrical means.

RESULTS

The chemical composition of the soil

The annual mean values of the levels of several water extractable nutrients in the soil are shown in Table 1. The forest soil contained significantly more extractable nutrients, particularly ammonium and sulphate, than the non-forested soil. The extractable amounts of many nutrients were significantly smaller in the forest soil in the coastal area than in the forest soils in other areas. However, the availability of sodium and chloride was largest in soils in the coastal region. The same trends were observed in the soils of the non-forested sites, but the differences were smaller and often not significant.

The extraction with a 0.2 N NaCl solution resulted in a higher yield of all nutrients than did the extraction with water (Table 2). This increase was extremely high for the base cations, calcium and magnesium, in the coastal region. In this region, the forest soil contained significantly less ammonium and aluminium than the forest soils in other areas. In the soil of the non-forested sites only the amount of extractable aluminium was significantly lower at the Coast.

Mineral balance and deposition

The results of the Pearson test are presented in Table 3. The nitrogen content and the NH_4/K and NH_4/Mg. ratio in the soil showed strong, positive correlation with the ammonium deposition and with the ammonium/cation ratios in the deposition. Both ammonium/cation ratios in the soil showed hardly any correlation with the absolute cation fluxes in the deposition.

The regional mean values of several ratios in the soil of the non-forested and forested sites and in bulk precipitation and throughfall deposition respectively, are presented in Table 4. At the coast the ammonium/cation ratios in throughfall deposition and in the forest soil were significantly lower than in other areas. However, in bulk deposition and soil extracts of the non-forested sites regional differences were smaller than in throughfall water and forest soil and, except for

Table 1: Annual (November 1986-1987) mean values of the levels of water extractable nutrients in μmol.100 g DW^{-1} on a regional and national scale (based on 14 locations in The Netherlands) in the soil of the forested and non-forested sites. Different letters within each line indicate statistical difference between the regions at the 5 % level according to Scheffé's multiple-comparison procedure.

	Coast		North		Centre		South		National
non-forested sites									
n	2		4		3		5		14
NH4	2	a	3	a	5	a	3	a	4
NO3	1	a	4	b	2	ab	2	ab	2
H	7	a	16	a	10	a	7	a	10
PO4	1.2	a	0.9	ab	0.4	b	1.1	a	0.9
SO4	4	a	4	a	6	b	6	ab	5
Cl	22	a	16	a	11	a	13	a	15
Na	21	a	12	ab	8	a	11	ab	12
K	5	a	5	a	7	a	6	a	6
Mg	1	a	1	a	1	a	1	a	1
Ca	1	a	2	a	2	a	2	a	2
Al	3	a	8	b	10	b	8	b	7
Mn	0.04	a	0.03	a	0.11	b	0.07	bc	0.06
Fe	2	a	2	a	4	a	3	a	3
Zn	0.1	a	0.1	a	0.1	a	0.1	a	0.1
forested sites									
n	4		11		8		13		36
NH4	4	a	16	b	24	bc	28	c	18
NO3	1	a	7	b	8	b	8	b	6
H	14	a	24	b	27	b	31	b	25
PO4	1.3	a	2.1	a	0.5	b	1.5	a	1.3
SO4	12	a	12	a	21	b	24	b	16
Cl	54	a	33	a	23	b	29	b	31
Na	54	a	18	b	16	b	22	b	21
K	7	a	8	ab	10	b	8	ab	8
Mg	2	a	2	a	3	b	3	b	2
Ca	1	a	3	b	3	bc	5	c	3
Al	6	a	14	b	18	c	16	bc	14
Mn	0.03	a	0.07	a	0.20	a	0.13	a	0.10
Fe	4	a	3	a	6	b	3	a	3
Zn	0.1	a	0.1	ab	0.2	bc	0.3	c	0.2

NH_4/Mg ratios in bulk deposition, not significant.

Although the NH_4/NO_3 ratios in the deposition also showed the lowest values in the coastal region, no such trend could be observed for the ratios in the soil. In both compartments ammonium dominated over nitrate.

Table 2: Annual (November 1986-1987) mean values of the levels of salt extractable nutrients in µmol.100 g DW^{-1} on a regional and national scale (based on 14 locations in The Netherlands) in the soil of the forested and non-forested sites. For the explanation of significance symbols see table 1.

	Coast		North		Centre		South		National
non-forested sites									
n	2		4		3		5		14
NH4	4	a	5	a	7	a	7	a	6
H	97	a	122	a	65	a	75	a	91
K	12	ab	10	a	19	a	16	ab	13
Mg	15	a	5	b	6	ab	9	ab	8
Ca	27	a	17	a	22	a	42	a	26
Al	18	a	62	b	70	b	41	b	47
Mn	0.5	a	0.3	a	0.7	a	0.3	a	0.4
Fe	0.7	a	0.4	a	0.7	a	0.5	a	0.5
Zn	0.8	a	0.5	a	0.7	a	0.9	a	0.7
forested sites									
n	4		11		8		13		36
NH4	7	a	28	b	44	b	46	b	32
H	173	a	179	a	143	a	175	a	171
K	15	a	17	a	21	a	15	a	17
Mg	28	a	10	b	10	b	11	b	11
Ca	38	ab	33	ab	29	a	50	b	38
Al	21	a	71	b	88	b	77	b	67
Mn	0.8	ab	0.6	a	1.1	b	0.8	ab	0.8
Fe	0.9	ab	0.5	c	1.2	a	0.7	c	0.7
Zn	0.8	a	0.7	a	0.8	a	1.6	b	1.0

Table 3: Correlation coefficients according to the Pearson-test between throughfall deposition (annual total) and water extractable nutrients in the soil (annual mean) of the forested sites (n=36): *,$P < 0.05$; **,$P < 0.01$; ***,$P < 0.001$.

SOIL	DEPOSITION						
	NH_4	NO_3	H	K	Mg	NH_4/K	NH_4/Mg
NH4	0.67***	0.10	-0.46**	0.11	-0.46**	0.59***	0.75***
NO3	0.62***	0.02	-0.43**	-0.04	-0.43**	0.65***	0.70***
H	0.30	0.04	-0.04	-0.06	-0.36*	0.35*	0.40*
K	0.16	-0.20	-0.19	-0.03	-0.24	0.12	0.28
Mg	0.12	0.15	-0.09	-0.08	-0.11	0.19	0.04
NH4/K	0.64***	0.19	-0.41*	0.12	-0.39*	0.58***	0.68***
NH4/Mg	0.53***	0.03	-0.47**	0.14	-0.48**	0.43**	0.65***

Table 4: Annual regional mean ratios in bulk and throughfall deposition and soil water extracts of non-forested and forested sites respectively. Different letters within each row indicate statistical difference between the regions at the 5 % level according to Scheffé's multiple-comparison procedure.

	n	NH_4/NO_3		NH_4/K		NH_4/Mg	
		deposition	soil	deposition	soil	deposition	soil
non-forested sites							
Coast	2	1.5a	2.2a	4.8a	0.4a	3.8a	2.3a
North	4	2.4b	1.0a	7.1a	0.7a	10.9ab	3.1a
Centre	3	2.5b	2.8a	6.4a	0.6a	14.1b	3.2a
South	5	2.6b	1.5a	5.3a	0.6a	12.9b	3.5a
Corsican pine sites							
Coast	2	1.4a	4.8a	2.5a	0.6a	1.4a	1.9a
North	4	6.0b	1.1b	5.8b	1.5b	20.3b	8.0b
Centre	3	4.2b	2.8ab	5.4b	1.8bc	25.1b	8.6b
South	4	4.7b	4.0a	6.3b	3.2c	15.8b	9.9b
Scots pine sites							
Coast	2	1.9a	6.5a	1.9a	0.6a	2.1a	2.6a
North	4	5.3b	2.2bc	4.8b	1.9b	17.2b	9.7b
Centre	2	4.8b	1.8c	5.2b	2.5b	21.3b	11.3b
South	5	5.2b	5.5ab	6.7b	2.8b	18.6b	11.6b
Douglas fir sites							
North	3	3.8a	4.3a	5.1a	2.9a	13.5a	9.5a
Centre	3	4.7a	4.2a	7.9b	3.1a	23.4a	6.6a
South	4	4.6a	2.0a	6.2b	5.1a	18.1a	8.7a

Mineral balance and nitrification

Table 5 shows that, in 33% of the forest soils the NO_3/Cl ratio increased in relation to the NO_3/Cl ratio in the deposition indicating a considerable nitrification (nitrification rate greater than tree uptake). In 67% of the forest soils nitrification rates were probably very low, although some tree uptake may be involved. The same calculations were made on a seasonal basis to estimate whether high temperatures in summer cause higher nitrification rates. The ratios and probably also the nitrification rates were hardly influenced by the seasons (data not shown).

To investigate the impact of nitrification on the mineral balance of the soil, the ammonium/cation ratios in soil and throughfall deposition were also compared. In 11 out of 24 stands with low nitrification rates both ammonium/cation ratios in the soil were more than 25 % higher than these ratios in the throughfall while this was the case in 10 out of 12 stands with high nitrification rates. In more than 50% of the soils with low nitrification rates the amount of ammonium deposited accounted directly for the ammonium/cation ratio in the soil.

Table 5: The relation between the nitrification rate and the mineral balance in the soil of the forested sites. (*) These values indicate the number of forest sites in which the soil NH_4/Mg and/or NH_4/K ratio were less or greater than 25 % higher than the same ratio in canopy throughfall; (**) Low or high nitrification: then soil NO_3/Cl is less or greater than NO_3/Cl in canopy throughfall.

	low nitrification	high nitrification (**)
number of locations	24	12
NH_4/Mg and NH_4/K <25% (*)	1	0
NH_4/Mg or NH_4/K <25% (*)	12	2
NH_4/Mg and NH_4/K >25% (*)	11	10

DISCUSSION

Under natural conditions nitrogen is considered the limiting factor in forests. At present, however, high nitrogen input to ecosystems causes disruption of the nitrogen cycle and nitrogen becomes easily available to the vegetation. Investigations in the Netherlands (Roelofs *et al.*, 1985; Verhoef & Dorel, 1988) and in Belgium (De Temmerman *et al.*, 1988) demonstrated that in areas with high emission of ammonia the amount of nitrogen in soils and trees is larger than in areas without emission sources.

In Dutch forests ammonium fluxes were five times as large as fluxes of nitrate (Houdijk & Roelofs 1991). According to Van Breemen *et al.* (1982) these increased ammonium fluxes cause severe acidification as a result of nitrification. They found that woodland soils contained more nitrate than ammonium. Kleijn *et al.* (1988) also measured nitrate dominance in the soil of Douglas fir stands. Indeed, several investigators have proved nitrification is possible in acid soils (Kriebitzsch, 1978; Alexander, 1980; Vonk *et al.*, 1988; De Boer *et al.*, 1988; Killham, 1990). However, the present research showed ammonium dominated nitrate in the top layer (15 cm) of the mineral soil in all of the investigated coniferous forests. The same dominance was observed by Van Dijk & Roelofs (1988) in stands of Scots pine. In the present study estimations of the nitrification rates showed that in 67 % of the forest soils this rate was probably either very low or negligible.

The aim of this research was to study the effect of an enhanced ammonium input on the mineral balance of the soil (Figure 1). This mineral balance showed a strong correlation with the ammonium flux and ammonium/cation ratios in the deposition. Comparison of soils with different nitrification rates clearly demonstrated that the ammonium/cation ratios in soils with low nitrification rates more often (13 out of 24) reflected the ratios in the deposition than in soils where part of the ammonium was converted to nitrate (2 out of 12 sites). No soils with high nitrification rates were studied in this research In such soils high ammonium/cation ratios are probably exceptional and acidification and concomitant processes like aluminium dissolution are of more concern (Van Breemen *et al.*, 1983).

Earlier research proved that the relative nutrient contents in the soil have more impact on growth and vitality of coniferous species than the absolute amount of nutrients (Roelofs *et al.*, 1985). Roelofs *et al.* (1985) measured NH_4/K and NH_4/Mg ratios in the soil and estimated tree vitality in stands of Corsican pine and Douglas fir. That field study clearly demonstrated a correlation between the mineral balance in the forest soil and the condition of trees. Less vital forests were often found on soils with high NH_4/Mg and/or NH_4/K ratios. Consequently critical values for these ratios were established at 5 and 10 respectively. A greenhouse experiment with Corsican pine, Scots pine and Douglas fir confirmed that increased ammonium fluxes to soils with relatively low nitrification rates lead to enhanced ammonium/nitrate as well as ammonium/cation ratios (Van Dijk *et al.*, 1989). In a soil with a high nitrification rate the ammonium/nitrate and ammonium/cation ratios were much lower. The needles of trees grown on the soil in which ammonium accumulated contained less potassium, magnesium and calcium (Van Dijk *et al.*, 1990). Laboratory experiments with young Corsican pine trees on hydrocultures showed that application of ammonium resulted in an increased nitrogen content whereas particularly the magnesium and calcium contents of the trees decreased (Boxman *et al.*, 1991).

The present study showed that NH_4/Mg ratios in the top soil layer more frequently exceed the critical value of 10 than NH_4/K the critical value of 5 (Table 4). The critical value of the NH_4/Mg was exceeded in 50% of all stands investigated. NH_4/K only became critical in Douglas fir stands at 50% of the sites (data not shown). Higher ratios in soils of Douglas stands were probably related to higher ammonium fluxes in these stands (Houdijk & Roelofs, 1991). Kleijn *et al.* (1988) also found a correlation between the vitality of Douglas fir and the NH_4/K ratio in the soil water. In their research the critical value was exceeded in 25% of the stands.

In conclusion, high nitrogen deposition, particularly ammonium deposition, accounted directly for the high ammonium content and ammonium/cation ratios in the soil. Nitrification rates in the top layer of the mineral soil were probably low in 67 % of the stands. In those stands the ammonium/cation ratios in the soil often reflected ammonium/cation ratios in throughfall deposition. However, even in stands with higher nitrification rates, ammonium remained the dominant compound of inorganic nitrogen in the top 15 cm of the soil. Generally the NH_4/Mg ratios more often exceeded the critical value than NH_4/K ratios in the top soil layer.

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CHAPTER 5

THE EFFECTS OF ATMOSPHERIC NITROGEN DEPOSITION AND SOIL CHEMISTRY ON THE NUTRITIONAL STATUS OF *PSEUDOTSUGA MENZIESII*, *PINUS NIGRA* AND *PINUS SYLVESTRIS*

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ABSTRACT

The ammonium content and the base cation content, expressed relative to ammonium, are enhanced in the soil of Dutch forests, due to the extremely high deposition of ammonium to the forest floor. A nation-wide investigation was carried out to establish whether and how these changed nitrogen fluxes in deposition and soil affect the nutritional status of coniferous trees.

The chemical composition of needles of Douglas fir, Scots pine and Corsican pine showed a regional trend similar to that of deposition and soil solution. Particularly nutrients, expressed relative to nitrogen, decreased from North to South. Of the macronutrients phosphorus was most often deficient and therefore probably limiting in the Douglas fir stands. Many pine trees suffered from relative magnesium shortages. In all stands magnesium and, in Douglas stands, also phosphorus contents of the needles were negatively correlated with ammonium and ammonium/cation ratios in deposition. However, in contrast to pine trees, nutrient contents in needles of Douglas fir showed correlation with nitrate rather than with ammonium in the soil solution. Correlation analyses indicate that nitrogen fluxes in the soil, indirectly affect the nutritional status of coniferous trees.

INTRODUCTION

A recent nation-wide survey revealed that almost 50 % of the Dutch forests show a decreased vitality (State Forest Service, 1990a). In the Netherlands enhanced nitrogen deposition is considered to play a major role in forest deterioration (Roelofs *et al.*, 1985).

Nitrogen generally is the limiting factor for biomass production in forest ecosystems. Fertilization experiments in several coniferous forests demonstrated that nitrogen application initially leads to an enhanced biomass production and nitrogen content of plant tissue. However, an increased nitrogen supply eventually leads to a relative shortage of other nutrients, as a result of which biomass production decreases again (Aronsson, 1985; Nihlgård, 1985; Nambiar & Fife, 1987; Oren *et al.*, 1988). The present nitrogen load in Dutch forests amounts to 80 kg $N.ha^{-1}.yr^{-1}$ (Houdijk & Roelofs, 1991) and clearly exceeds the amount of nitrogen needed to keep up biomass production in forests (5-8 kg $N.ha^{-1}.yr^{-1}$ according to Encke (1986)). Phosphorus deficiency (Mohren *et al.*, 1988) and magnesium and potassium deficiency (Roelofs *et al.*, 1985) have been associated with nitrogen fluxes in Dutch forests. The latter are easily recognized by yellowing of the needles. Yellowing of current needles is accompanied by arginine accumulation; this phenomenom is probably also associated with an enhanced nitrogen input (Van Dijk & Roelofs, 1988). Greenhouse experiments in which several coniferous trees were treated with ammonium-enriched rain water showed that the nutritional status of the soil and the trees changed. The earlier mentioned phenomena accompanied these mineral disturbances (Van Dijk *et al.*, 1989 & 1990).

This study was carried out to investigate whether and how the present high nitrogen fluxes to Dutch forests affect the nutritional status of three coniferous tree species. In this paper the results of a nation-wide investigation are presented. The chemical composition of needles of 36 coniferous trees at 14 locations was determined and evaluated according to nutrient thresholds known from literature. Furthermore, the relation between the nutritional status of current needles and the chemical composition of the forest soil and throughfall deposition was studied.

MATERIALS AND METHODS

In November 1986 36 forested sites at 14 locations in the Netherlands were selected. The investigation concerned 13 Corsican pine (*Pinus nigra* var. *maritima* (Ait.) Melville), 13 Scots pine (*Pinus sylvestris* L.) and 10 Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands. The selection of the locations was primarily based on the occurrence of all three coniferous tree species within a distance of 5 km from each other. Furthermore, 25-40-year-old stands were chosen to diminish variations caused by age differences. Neither tree vitality nor the occurrence of illnesses or infection were considered when selecting the stands. After selection of the stands, the locations were grouped into four regions according to their geographical situation; Coast, North, Centre and South. The chemical composition of deposition fluxes at these sites has been described by Houdijk & Roelofs (1991). In Houdijk *et al.* (1993) the distribution of the sampling sites and detailed information about the chemical composition of the soil solution can be found.

After selection of the sites one representative tree was chosen for sampling in each stand. Every two months, soil samples and every month, deposition samples were collected underneath these trees during 18 months. At each of the 14 locations soil and deposition were also sampled at one open, treeless site. needle samples were collected after one year, in November 1987. Needles were taken from several first and second order branches from the middle of the crown. In stands of Corsican pine and Scots pine 6 and 18-month-old needles were gathered. In Douglas fir stands 6 and 30-month-old needles were sampled. Needle analyses were carried out as described by Van Dijk & Roelofs (1988).

Scheffé's multiple-comparison procedure (performed with the General Linear Models (GLM) procedure) was used to establish the impact of regional variability on the nutritional status of the needles. The paired t-test procedure was used as a test of significance for differences between means. The Pearson test was applied to determine the relation between the nutritional status of the trees and the chemical composition of throughfall fluxes (annual total) and forest soil solution (annual mean) (SAS Institute Inc., 1985). Prior to statistical analysis the data were log-transformed to make the variance independent of the mean (Sokal & Rohlf, 1981). For presentation the data were backtransformed. Consequently, the mean values given are geometrical means.

RESULTS

The chemical composition of current needles for each tree species per region is shown in Table 1. Most of the nutrient contents, relative and absolute, measured in needles of all tree species showed no significant regional differences. However, a trend in nutrient contents expressed relative to nitrogen could be observed. Relative nutrient contents decreased from North to South. This trend was also found for the absolute magnesium content of all tree species. Furthermore, the absolute phosphorus and potassium content were lowest in needles of Scots pine and Douglas fir, respectively, in the southern region. Only, in the needles of Scots pine at the Coast the relative magnesium content was significantly higher than in other areas in the Netherlands.

Older needles of all tree species contained significantly less phosphorus and potassium than 6-month-old needles (Table 2). The magnesium content also decreased with age, though only significantly in pine needles. Nitrogen and calcium, however, tended to accumulate in older needles. In all stands phosphorus, magnesium and potassium contents relative to N (N=100%) were significantly lower in older needles. The same trends could be observed in Scots pine stands, although differences were not always significant.

In Table 3, nitrogen contents in current needles of the trees are compared to normal ranges of nitrogen contents used to define deficiency, normal and excess concentrations (State Forest Service, 1990b). Needles of Scots pines showed the highest N-contents and in 10 out of 13 stands the optimum value of 1.8 % N was exceeded. Current needles of two Corsican pine stands and two Douglas fir stands were N-deficient whereas at three and four sites respectively, high N values were measured.

In Table 4, the nutritional status of the trees is compared with threshold values of several other nutrients (State Forest Service, 1990b). Phosphorus was critical in many Corsican pine and Douglas fir stands, but only two Scots pine stands contained low amounts of phosphorus. At 50 % of all the Douglas fir sites but in only one pine stand, potassium levels were below the critical value. However, magnesium and

Table 1: Chemical composition of 6 month-old needles of Corsican pine, Scots pine and Douglas fir. Nutrients in % of DW and ratios in % of N-content (when N=100), mean values per region. Different letters within each column indicate statistical difference between the regions at the 5% level according to Scheffé's multiple- comparison procedure.

	n	N	P	K	Mg	Ca	P:N	K:N	Mg:N
Corsican pine									
Coast	2	1.03a	0.10a	0.62a	0.07a	0.13a	10.0a	60.2a	6.7a
North	4	1.64a	0.13a	0.77a	0.06a	0.06a	7.9a	47.3a	3.4a
Centre	3	1.45a	0.11a	0.89a	0.07a	0.08a	7.7a	61.3a	4.8a
South	4	1.62a	0.12a	0.64a	0.05a	0.13a	7.3a	39.2a	3.1a
Scots pine									
Coast	2	1.75a	0.16a	0.60a	0.11a	0.30a	9.3a	34.6a	6.4a
North	4	1.87a	0.16a	0.65a	0.07ab	0.28a	8.6a	34.9a	3.9b
Centre	2	2.22a	0.17a	0.75a	0.07ab	0.15a	7.7ab	33.8a	3.1b
South	5	1.99a	0.12a	0.72a	0.06b	0.21a	6.3b	36.4a	2.8b
Douglas fir									
North	3	1.44a	0.12a	0.63ab	0.13a	0.37a	8.1a	43.6a	8.8a
Centre	3	1.74a	0.09a	0.76a	0.11a	0.33a	4.9a	43.5a	6.3a
South	4	2.06a	0.10a	0.48b	0.08a	0.41a	4.9a	23.3a	4.1a

Table 2: Chemical composition of 6 and 18-(or 30) month-old needles of Corsican pine, Scots pine and Douglas fir. Nutrients in % DW and ratio in % of N content (when N=100), mean values per tree species. Statistical differences between current and old needles. Significance according to the paired t-test; *, P<0.05; **, P<0.01; ***, P<0.001.

	Corsican pine n=12			Scots pine n=11			Douglas fir n=10		
	6-mth needles	18-mth needles		6-mth needles	18-mth needles		6-mth needles	30-mth needles	
N	1.52	1.57		1.95	1.93		1.73	2.42	**
Mg	0.06	0.04	***	0.07	0.06	*	0.10	0.08	
K	0.75	0.56	**	0.67	0.48	***	0.60	0.33	***
Ca	0.09	0.11	**	0.22	0.33	**	0.34	0.45	***
Fe	0.01	0.02	*	0.01	0.02		0.03	0.02	
Al	0.02	0.04	*	0.01	0.02		0.02	0.02	
P	0.12	0.09	**	0.15	0.13	**	0.10	0.07	*
Mg:N	3.9	2.8	**	3.5	2.9	*	5.7	3.3	***
K:N	49.6	35.4	**	34.2	24.8	**	34.5	13.7	***
P:N	7.8	6.0	***	7.7	6.5	**	5.5	2.9	***

Table 3: Deficiency, normal and excess N-levels (% DW) for Corsican pine, Scots pine and Douglas fir (State Forest Service, 1990b) and the number of trees (n) with N-contents in needles within the range.

	Corsican pine n=13		Scots pine n=13		Douglas fir n=10	
	N	n	N	n	N	n
deficiency	<1.3	2	<1.4	-	<1.4	3
normal	1.3-1.8	8	1.4-1.8	3	1.4-1.8	3
excess	>1.8	3	>1.8	10	>1.8	4

Table 4: Threshold values of nutrient concentrations and ratios for nutrient deficiency (tc) of P, K, Mg en Ca (in % DW and % of N-content) (State Forest Service, 1990b) and number of trees (n) with nutrient values below the threshold values for normal growth of Corsican pine, Scots pine and Douglas fir.

	Corsican pine n=13		Scots pine n=13		Douglas fir n=10	
	tc	n	tc	n	tc	n
P	0.13	8	0.13	2	0.14	9
K	0.50	1	0.50	-	0.60	5
Mg	0.06	4	0.07	5	0.07	2
Ca	0.10	6	0.20	5	0.20	1
P:N	5	-	5	-	5	3
K:N	25	-	25	1	25	3
Mg:N	5	10	5	9	5	3

calcium deficiencies were more frequently measured in pine trees than in firs. Magnesium levels relative to nitrogen were critical in 19 out of 26 pine stands. Relative phosphorus deficiency did not occur in pine stands and relative potassium deficiency was established in only one pine stand. Relative magnesium, potassium and phosphorus deficiencies occurred in 3 out of 10 Douglas fir stands.

The Pearson correlation test was used to study the correlation between the nutritional status of the needles and the chemical composition of the deposition (Table 5). Deposition parameters which were tested are ammonium, nitrate, ammonium/nitrate, acid, phosphorus, potassium, ammonium/potassium, magnesium, ammonium/magnesium and calcium. Only the parameters which showed statistical significant correlations are presented in Table 5. Nitrogen content of pine trees, in contrast to firs, was positively correlated with ammonium and the ammonium/nitrate

Table 5: Pearson correlation coefficients between nutrients and ratios in 6 months-old needles and throughfall deposition (annual total) of Corsican pine, Scots pine and Douglas fir stands. Significance according to Pearson: (*), P<0.10; *, P<0.05; **, P<0.01; ***, P<0.001.

DEPOSITION	NEEDLES							
	N	K	K:N	Mg	Mg:N	P	P:N	Ca
Corsican pine (n=13)								
NH4	0.69 **	0.48 (*)	-0.19	-0.50 (*)	-0.77**	0.13	-0.66 *	-0.14
NH4/NO3	0.84***	0.40	-0.40	-0.37	-0.75**	0.27	-0.71**	-0.44
H	-0.72**	-0.50 (*)	0.19	0.23	0.57 *	-0.18	0.65 *	0.56 *
P	0.64 *	0.42	-0.21	-0.20	-0.51 (*)	0.18	-0.57 *	-0.03
NH4/K	0.43	0.46	0.02	-0.31	-0.47	-0.01	-0.48 (*)	-0.34
Mg	-0.50 (*)	-0.23	0.24	0.08	0.34	-0.12	0.45	0.47
NH4/Mg	0.70**	0.45	-0.22	-0.31	-0.62 *	0.12	-0.67 *	-0.41
Ca	-0.18	-0.13	0.04	-0.16	-0.02	-0.30	-0.06	0.56 *
Scots pine (n=13)								
NH4	0.55 *	0.01	-0.44	-0.43	-0.57 *	-0.07	-0.51 (*)	-0.18
NH4/NO3	0.46	0.11	-0.25	-0.55 (*)	-0.65 *	-0.05	-0.42	-0.28
NH4/K	0.59 *	0.28	-0.18	-0.58 *	-0.72**	-0.14	-0.61 *	-0.33
Mg	-0.28	-0.18	0.03	0.60 *	0.63 *	0.07	0.30	0.46
NH4/Mg	0.53 (*)	0.12	-0.30	-0.69**	-0.79**	-0.12	-0.54 (*)	-0.45
Douglas fir (n=10)								
NH4	0.21	-0.21	-0.23	-0.49	-0.47	-0.79**	-0.63	-0.30
NH4/NO3	0.09	-0.20	-0.16	-0.25	-0.22	-0.62 (*)	-0.45	-0.22
NH4/K	0.68 *	-0.43	-0.61 (*)	-0.61 (*)	-0.74 *	-0.59 (*)	-0.78**	-0.23
NH4/Mg	0.31	-0.22	-0.29	-0.65 *	-0.60 (*)	-0.82**	-0.70 *	-0.53

ratio in the deposition. The nitrogen content and also the relative magnesium and phosphorus contents in needles of Corsican pine were correlated with ammonium (positively and negatively respectively) and acid fluxes (negatively and positively respectively). In needles of Scots pine the nitrogen content showed positive correlation and relative magnesium and phosphorus contents showed negative correlations with ammonium, ammonium/magnesium, ammonium/potassium and ammonium/nitrate ratios in the deposition. In needles of Douglas fir nitrogen content was positively and relative potassium, magnesium and phosphorus content were negatively correlated with one or both ammonium/cation ratios in the deposition. The phosphorus content in fir needles was negatively correlated with ammonium, the ammonium/cation and ammonium/nitrate ratios in the deposition.

The soil parameters which were tested are similar to the deposition parameters extended with aluminium. In Table 6, only the soil parameters which showed significant correlations are presented. By comparison of the nutritional status of the needles and the extractablity of nutrients in the soil, nitrogen contents in needles of all trees showed positive correlation with the nitrate content and negative correlation with the ammonium/nitrate ratios in the soil. Only in needles of Corsican pine was nitrogen positively correlated with ammonium and both ammonium/cation ratios in the soil. The relative potassium, magnesium and phosphorus contents of pine needles were often negatively correlated with ammonium and ammonium/cation ratios in the soil. In needles of Corsican pine, the strongest negative correlation with both ratios was established for relative phosphorus contents, whereas in Scots pine needles, relative potassium and magnesium contents were best correlated with the ammonium/magnesium and ammonium/potassium ratios respectively. In contrast to pine trees, relative and absolute nutrient contents in needles of Douglas firs only showed positive correlation with nitrate rather than with ammonium. However, in firs, several nutrient contents, absolute as well as relative, showed correlation with ammonium/nitrate but neither with the ammonium/potassium nor with the ammonium/magnesium ratio in the soil.

Table 6: Pearson correlation coefficients between nutrients and ratios in 6 months-old needles and soil water extracts (annual mean) of Corsican pine, Scots pine and Douglas fir stands. See table 5 for significance symbols.

SOIL	NEEDLES							
	N	K	K:N	Mg	Mg:N	P	P:N	Ca
Corsican pine (n=13)								
NH4	0.77 **	0.20	-0.50(*)	-0.29	-0.65 *	0.12	-0.75**	-0.20
NO3	0.73 **	0.53(*)	-0.19	-0.34	-0.67 *	0.48(*)	-0.41	-0.24
P	0.46	0.25	-0.19	0.01	-0.25	0.62 *	0.01	0.16
NH4/K	0.85***	0.30	-0.49(*)	-0.08	-0.54(*)	0.23	-0.76**	-0.22
NH4/Mg	0.77**	0.19	-0.51(*)	-0.38	-0.72 **	0.04	-0.82***	0.01
Ca	0.36	-0.05	-0.36	-0.55(*)	-0.62 *	-0.11	-0.49(*)	-0.07
Scots pine (n=13)								
NH4	0.37	-0.05	-0.36	-0.57 *	-0.63 *	-0.15	-0.45	-0.28
NO3	0.58 *	0.11	-0.35	-0.26	-0.44	0.28	-0.15	-0.27
NH4/NO3	-0.39	-0.11	0.20	0.17	-0.01	-0.54(*)	-0.27	0.17
H	0.20	-0.02	-0.19	-0.06	-0.13	-0.37	-0.56 *	0.23
NH4/K	0.44	-0.24	-0.63 *	-0.48(*)	-0.58 *	-0.05	-0.41	-0.26
Mg	-0.24	0.41	0.64 *	0.13	0.20	-0.08	0.10	0.01
NH4/Mg	0.58 *	0.09	-0.38	-0.69**	-0.81***	-0.05	-0.52(*)	-0.51(*)
Al	0.14	0.40	0.33	-0.49(*)	-0.48(*)	-0.23	-0.35	-0.26
Douglas fir (n=10)								
NO3	0.57(*)	-0.68 *	-0.68 *	-0.67 *	-0.73 *	-0.41	-0.60(*)	-0.16
NH4/NO3	-0.80 **	0.72 *	0.83 **	0.64 *	0.81 **	-0.01	0.47	0.17
H	0.41	-0.44	-0.47	-0.10	-0.26	-0.56(*)	-0.60(*)	0.07
Ca	0.65 *	-0.41	-0.58(*)	-0.20	-0.43	-0.20	-0.51	0.27
Al	0.61(*)	0.02	-0.32	-0.38	-0.54	-0.24	-0.52	-0.09

The nitrogen content in needles of Douglas fir was significantly, negatively correlated with phosphorus, as well as potassium and magnesium contents. The nitrogen content in needles of pine trees was significantly, negatively correlated with calcium and in needles of Corsican pine also, weakly, with magnesium (Table 7).

Table 7: Pearson correlation coefficients between N-content and P, K, Mg and Ca-content of needles of Corsican pine, Scots pine and Douglas fir. See Table 5 for significance symbols.

	Corsican pine	Scots pine	Douglas fir
P	0.23	-0.02	-0.45 *
K	-0.06	0.13	-0.80 ***
Mg	-0.28 (*)	-0.23	-0.41 (*)
Ca	-0.37 *	-0.42 *	0.10

DISCUSSION

Two previous studies showed that the coastal region was obviously least affected by high ammonium fluxes, both in deposition and soil solution (Houdijk & Roelofs, 1991; Houdijk *et al.*, 1993). In the other regions ammonium fluxes and related ammonium/cation ratios displayed an increasing trend from North to South. Similar regional variation was found in the present study. N-contents in needles of pine trees were lowest in the coastal region and in needles of Douglas fir in the northern region. In fact, the five stands with low N-contents (Table 3) are all found in those regions. Although absolute nutrient contents showed no regional variation, magnesium and phosphorus contents expressed relative to nitrogen, which according to Ingestad (1976) and Van den Burg (1990) may even be more important than absolute levels, showed a decreasing trend from North to South.

The relation between nitrogen deposition and the nutritional status of coniferous tree species was confirmed by findings of Van den Burg & Kiewiet (1989). In their study concerning trees in the south-eastern part of the Netherlands, they found that the increasing concentrations of livestock (c.q. the increasing ammonium deposition) were accompanied by an enhanced N-content in the needles of Scots pine and Douglas fir from 1.5 and 1.4 %, respectively, to 2.2 %. Such high N-levels in current needles of Douglas fir were only reached in the southern region. Studies of Oterdoom *et al.* (1987) and Evers *et al.* (1991) and also the present study showed that older needles contain more nitrogen than current ones. According to Van den Burg (1990) this phenomenon may also be ascribed to the extremely high nitrogen input into

Dutch forests, as in general older tissues contain less nitrogen than younger tissues.

High nitrogen contents are probably not directly associated with needle damage but rather cause other nutrients to become deficient (Van den Burg, 1988; Evers *et al.*, 1991). According to Van den Burg (1990) the increasing N deposition in the south-eastern part of the Netherlands is largely responsible for the present absolute and relative phosphorus deficiencies in current needles of Douglas firs whereas in pine needles the calcium and absolute and relative magnesium contents are mostly affected. The present study showed that phosphorus deficiencies occurred most frequently. In particular, Douglas fir suffered from absolute and relative phosphorus deficiencies, but also from absolute potassium deficiencies. In contrast to firs, however, pine trees often showed calcium and absolute and relative magnesium shortages. This is in good agreement with findings of Van Dijk *et al.* (1990) who found that high ammonium application led to low potassium levels in needles of Douglas fir, whereas low magnesium and calcium levels were measured in pine trees.

Correlation analyses confirmed the relation between ammonium fluxes in deposition and soil solution and the nutritional status of the trees. Besides nitrogen, several other nutrients and, even more clearly, the relative nutrient contents of needles were more or less correlated with ammonium fluxes and ammonium/cation and ammonium/nitrate ratios in deposition. The nutritional status of pine trees seemed to be stronger correlated with ammonium fluxes and ratios in the soil solution than with those in deposition whereas nutrient contents of Douglas fir needles hardly showed any correlation with ammonium fluxes and ammonium/cation ratios in the soil solution and in deposition. In contrast to pine trees, nutrients in needles of firs were correlated with nitrate fluxes and ammonium/nitrate ratios in the soil solution. These differences indicate that the deposition of ammonium affects the nutritional status of the trees investigated mainly indirectly, via the soil compartment. The results of a greenhouse experiment, in which two watering methods were used, indicated that uptake of ammonium by the canopy was probably negligible (Van Dijk *et al.*, 1990). In that experiment the chemical composition of trees which received ammonium by pouring water over the tree crown did not differ from that of trees to which the ammonium enriched water was applied indirectly, via the soil compartment. Correlation analyses indicate that Douglas firs, in contrast to pine trees, prefer nitrate to ammonium. Krajina *et al.* (1973) indeed found that Douglas fir grew better on soils in which nitrification took place. Also many laboratory experiments showed that Douglas firs prefer nitrate nutrition (Krajina *et al.*, 1973; Gijsman, 1990) while several pine species prefer ammonium or a mixture of ammonium and nitrate (Nelson & Selby, 1974; Scheromm *et al.*, 1990; Mcfee & Stone, 1968). Nitrate nutrition stimulates cation uptake (Krajina *et al.*, 1973; Nelson & Selby, 1974; Scheromm *et al* ., 1990) and depresses phosphorus uptake whereas ammonium nutrition stimulates phosphorus uptake (Bledsoe & Zasoski, 1983; Salsac *et al.*, 1987; Boxman & Roelofs,

1988) and depresses cation uptake (Morgan & Jackson, 1988; Boxman *et al.*, 1991). Correlation analyses (Table 7) indicated that nitrogen nutrition of Douglas fir affected the phosphorus content but also the cation contents of the needles. Nitrogen nutrition of pine trees affected the calcium content but seemed to have no stimulating effect on the phosphorus level of the needles. High nitrogen supply probably causes phosphorus deficiencies in Douglas firs, whereas in pine trees calcium shortages occur and this difference is probably connected with the fact that those tree species use different nitrogen forms. The preference of nitrate, however, cannot explain the high negative correlation between nitrogen and potassium in needles of Douglas fir, as nitrate nutrition is usually accompanied by cation uptake. Rygiewicz & Bledsoe (1984) also found that Douglas fir has very low rates of potassium uptake.

In conclusion: the nutritional status of needles of Douglas fir, Scots pine and Corsican pine was clearly, and most probably, indirectly affected by the high ammonium fluxes in the deposition, via the soil compartment. Although regional variation in the chemical composition of needles was not as clear at that of deposition fluxes and soil solution the nutritional status of trees seemed more balanced in the less polluted part of The Netherlands. The differences in the nature of the deficiency in needles of Douglas fir and pine trees may be related to the different N-forms they use to meet their N-demand.

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CHAPTER 6

EFFECTS OF AMMONIUM DEPOSITION ON THE MINERAL BALANCE OF FOREST SOILS WITH DIFFERENT NITRIFICATION RATES

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ABSTRACT

Soil percolation experiments were used to study the effect of ammonium deposition in a staged gradient on the nutritional balance of forest soils differing in nitrification rates. In all soil types ammonium/cation and aluminium/calcium ratios increased when the amount of ammonium administered to the columns was larger than the ambient deposition level. At high deposition levels on low to moderately low nitrifying soils the threshold value of the ammonium/potassium ratio was exceeded first whereas in highly nitrifying soils the critical value of the aluminium/calcium ratio was reached first. When the columns received ammonium in amounts lower than the amounts deposited in the field the soils seemed able to restore their mineral balance. This study proved that soil percolation experiments can be used to predict the effects of increasing or decreasing ammonium deposition on nitrogen saturated forests despite the absence of vegetation. The presence of litter on intact soil cores leads to a higher nitrate production but does not change soils with low nitrification rates into soils with a high nitrifying capacity.

INTRODUCTION

In many West-European countries reduced nitrogen compounds contribute more to the total atmospheric nitrogen deposition than oxidized nitrogen compounds (Grennfelt & Hultberg, 1986). Particularly forest ecosystems suffer from high ammonium sulphate deposition due to the filtering action of the canopy. In the Netherlands throughfall fluxes of ammonium amount to 80 kg NH_4-N.ha^{-1}.yr^{-1} (Houdijk & Roelofs, 1991). These high ammonium fluxes to the forest soil disturb the mineral balance in the soil which in turn may cause a nutritional imbalance in the needles of coniferous trees (Schulze *et al.*, 1989; Van Dijk *et al.*, 1989, 1990; Houdijk *et al.*, 1993; Houdijk & Roelofs, 1993). Prolonged ammonium supply on the forest soil may lead to soil acidification due to nitrification and concomittant leaching of base cations and dissolution of aluminium (Van Breemen *et al.*, 1982; Ulrich, 1983). In soils with low nitrification rate high ammonium fluxes to the forest soil may lead to ammonium accumulation and leaching of base cations (Roelofs *et al.*, 1985).

In forest soils a combination of acidifying and eutrophicating soil processes can be expected. In the present study two soil-percolation experiments were conducted to investigate in what way and to what extent the nutritional balance of the soil can be affected by these two soil processes. The objective of the first experiment is to study the effect of ammonium deposition in a staged gradient on the nutritional balance and nitrification rate in the mineral soil of four coniferous forests. As according to Tietema *et al.* (1991) nitrification is mainly restricted to the organic ectolayer a second experiment was carried out. In this experiment a comparison was made between the nitrification rates in intact soil cores of low nitrifying soils with and without removal of the litter layer. During both column-experiments percolation water was sampled and analyzed for its chemical composition. From the comparison of ammonium/potassium and aluminium/calcium ratios in the effluent with their threshold values (5 and 1, respectively, according to Ulrich (1983), Roelofs *et al.* (1985) and Van Breemen & Verstraten (1990)) for each soil type that level of ammonium deposition was determined at which the mineral balance becomes disturbed. Furthermore, the influence of nitrification on this disturbance was studied. The influence of plant uptake was not taken into account as both column-experiments were carried out without vegetation.

MATERIAL AND METHODS

Experiment I

Soils were sampled in four Dutch pine stands near Budel, Orvelte, Hulst and Heumen. After removal of the litter layer the top 25 cm of the mineral soil was collected. After thoroughly mixing and sieving (2mm mesh sieve) the fresh soil was brought into PVC tubes (length 30 cm and inner diameter 7.5 cm). At the bottom of each tube a polyethylene, perforated cap, containing a small-mesh netting, was placed. The tube with cap was placed on a funnel which discharged into a 250 ml blackened polyethylene pot to collect percolation water. Each pot contained 0.5 ml of a fixating solution (200 mg $HgCl_2.l^{-1}$) to inhibit microbial activity. All columns were covered by a lid and held at a temperature of 20 °C.

The columns were percolated with an ammonium containing solution which resembled throughfall water qua pH (pH=5) and chemical composition but varied in ammonium sulphate content. Five days a week, 20 ml solution was added to the columns during 4 months. This way singular soil columns were infiltrated with 6 different solutions containing 100, 250, 500, 750, 1000 or 2500 μM $(NH_4)_2SO$&'(4). The ammonium deposition amounted to 11.2, 28, 56, 84, 112 and 280 kg NH_4-$N.ha^{-1}$ administered in 19 weeks. Furthermore the solutions contained 100 μM potassium, 100 μM magnesium, 200 μM calcium, 200 μM sodium, 100 μM nitrate and 500 μM chloride.

At regular intervals the effluent was analyzed for pH and chemical composition. Chemical analyses were performed according to Houdijk & Roelofs (1991).

Average net nutrient fluxes were calculated by multiplying weekly concentrations by the weekly volume of the effluent minus the amount weekly added to each column. Prior to statistical analysis, these values were log-transformed to make variance independent of the mean (Sokal & Rohlf, 1981). For presentation all data were transformed back and presented as geometrical means.

The influence of the experimental factors on the nutrient fluxes for each location was analyzed with two way analysis of variance (ANOVA) models including all first order interactions. The dependent variables were the weekly amount of water (ml) and nutrient fluxes (NH_4, NO_3, H, Al, Mg, Ca, K and H) and nutrient ratios (Al/Ca, NH_4/K and NH_4/Mg). The independent variables were ammonium treatment (six levels) and time (ten levels).

In addition, Tukey's multiple-comparison procedure was performed on soil type and ammonium treatment. The statistical analyses were performed with the General Linear Models (GLM) procedure available in the Statistical Analysis System (SAS) software package (SAS Institute Inc., 1985).

Experiment II

For the second experiment intact soil cores with and without litter layer from Hulst and Heumen were used. Twelve cores per location were collected by pushing PVC tubes (diameter 7.5 cm and length 30 cm) into the top soil. From 6 of the cores the litter layer was removed after collection. To minimize the role of spatial varability, all cores were taken from a small area (approximately 1 m^2). The experimental setup of the tubes was analogous to that of experiment I.

During 6 months and 5 days a week the soil cores were treated with 10 ml of 3 different solutions containing 250, 500 and 1000 µM $(NH_4)_2$SO&'(4). This resulted in an ammonium deposition of 14, 28 and 56 kg NH_4-N.ha^{-1} in half a year. In this experiment two replicates of each column were used. Concentrations of other elements in the solution applied were similar to those of experiment I.

In this experiment cumulative net fluxes of water and nutrients were calculated by summarizing all two-weekly fluxes and distracting the amount added during the experiment. The average values presented are geometrical means of two replicates.

The dependent variables in the ANOVA analysis per location were similar to those of experiment I. The independent variables were soil type, ammonium treatment and litter layer. Tukey's multiple-comparison procedure was consequently performed on soil type, ammonium treatment and litter layer.

RESULTS

Experiment I

Prior to two way analysis, three-way analysis of variance clearly demonstrated significant differences in the chemical composition of the percolation water between the four different soils. In Table 1 average net fluxes of water and nutrients weekly washed out of the soil columns and average weekly ratios are presented. The estimated mean amount of water retained weekly by the columns, was 94-95 ml for all soil types. All net nutrient fluxes and ratios in the Heumen soil were significantly different from those in the Budel soil. Net fluxes and ratios in both other soil types generally were intermediate though not always significantly different from those in the Heumen and Budel soil. Exceptions were found by H^+ and potassium net fluxes in the Orvelte soil and the ammonium net flux in the Hulst soil which were significantly highest and the aluminium flux which was significantly lowest in the Orvelte soil.

The influence of experimental factors clearly differed per soil type; therefore, the influences were investigated for each location separately. Table 2 shows per location whether net nutrient fluxes and ratios were affected by treatment, the duration of the treatment or a combination of both.

Table 1: The estimated average net fluxes of water (ml) and several nutrients (µmol) and ratios weekly washed out of the columns per location. Average values are geometrical means. Different letters within a row indicate statistical differences, at a 5 % level according to Tukey's multiple-comparison procedure. Positive fluxes indicate efflux from the column and negative fluxes indicate accumulation or conversion in the columns.

Location	Hulst	Heumen	Budel	Orvelte
ml	-94.7 a	-94.8 a	-94.8 a	-94.1 a
NO3	37.0 b	-0.7 c	150.3 a	32.9 b
NH4	10.8 a	-32.5 b	-246.0 c	-31.3 b
H	23.7 b	6.2 d	10.9 c	77.6 a
Al	17.9 b	8.0 c	19.3 a	6.1 d
K	2.5 b	2.0 b	-2.0 c	11.7 a
Mg	-1.8 b	-5.5 c	0.6 a	-2.9 b
Ca	7.8 b	-6.0 c	42.4 a	5.0 b
Al/Ca	1.0 b	1.8 a	0.4 c	0.4 c
NH4/K	6.3 a	5.7 a	0.2 c	3.6 b
NH4/Mg	17.4 b	22.0 a	0.4 d	14.0 c

In the **Hulst** soil all dependent variables but H^+ were significantly affected by the ammonium treatment but these differences varied during the experiment except for the aluminium fluxes. In fact the net H^+ flux showed no overall significant differences between treatments but note that time differences were not identical for all treatments. Most net nutrient fluxes and ratios from the columns filled with Hulst soil increased with the enhancing ammonium treatment (Table 3). Only the net

Table 2: P-values of ANOVA for the influence of the ammonium treatment (tr) and time (t) and the interaction between treatment and time (tr.t) on water and nutrient fluxes and ratios in the percolation water. P-values of the analysis of variance: *** $P \le 0.001$, ** $0.001 < P \le 0.01$, * $0.01 < P \le 0.05$.

Location	Hulst			Heumen			Budel			Orvelte		
	tr	t	tr.t	tr	t	tr.t	tr	t	tr.t	tr	t	tr.t
ml	**	**	-	-	-	-	-	-	-	-	-	-
NO3	***	***	***	-	*	-	***	**	-	-	-	-
NH4	***	***	***	***	***	***	***	***	***	***	**	**
H	-	***	*	-	-	-	***	-	*	-	*	-
Al	***	***	-	***	***	**	***	-	*	**	-	-
K	***	***	***	***	*	-	***	***	***	*	***	-
Mg	***	***	***	***	***	**	***	***	-	-	-	-
Ca	***	***	**	*	*	-	***	***	**	-	-	-
Al/Ca	**	***	*	*	-	-	***	***	***	-	-	-
NH4/K	***	***	**	***	***	-	***	***	*	***	***	**
NH4/Mg	***	***	**	***	***	*	***	***	*	**	-	-

ammonium efflux decreased and finally resulted in accumulation at the highest ammonium treatment whereas the nitrate efflux and the Al/Ca ratio fluctuated with treatment. However, the absolute efflux of ammonium increased with increasing deposition but is not large enough to prevent accumulation when the ammonium input is very large. Generally, net H^+, aluminium, potassium, magnesium and calcium fluxes from the column treated with the lowest ammonium deposition differed significantly from the one treated with the highest ammonium deposition. At all other treatments, net fluxes were intermediate but not always significantly different from those at the lowest or highest treatment. For net nitrate fluxes the influence of treatment is more complex. Figure 1 shows that nitrate fluxes at the 11.2 and 84 kg N load decrease more during the experiment than expected compared to the fluxes at the other treatments. Ammonium effluxes showed a clear gradient at the beginning of the experiment but towards the end ammonium effluxes at all treatments stabilized at zero. Only at the highest treatment ammonium accumulated from the beginning of the experiment and showed an increasing trend until ammonium accumulation seemed to stabilize at -200 μmol. The Al/Ca ratio clearly fluctuated with treatment as well as in time and no clear gradient could be observed. The NH_4/K and NH_4/Mg ratios both initially increased at all treatments. After about 8 weeks both ratios decreased at the three lowest, increased further at the highest and stabilized at both other treatments.

Table 3: The estimated net average nutrient fluxes (µmol) and ratios in the percolation water for six ammonium treatments (in kg NH_4- N.ha^{-1}.yr^{-1}) per soil type. Average values are geometrical means. Different letters within a row indicate statistical differences, at a 5 % level according to Tukey's multiple-comparison procedure. Positive fluxes indicate efflux from the column and negative fluxes indicate accumulation or conversion in the columns.

	ammonium treatment					
	11.2	28	56	84	112	280
Hulst						
NO3	36.4 bc	59.4 a	49.8 ab	18.9 d	31.0 c	26.2 cd
NH4	72.6 a	57.1 a	34.7 b	26.1 bc	9.3 c	-141.7 d
H	19.0 b	23.7 ab	24.2 ab	22.4 ab	23.5 ab	29.9 a
Al	15.5 d	16.0 cd	16.8 bcd	18.2 bc	19.0 b	24.1 a
K	-2.3 d	2.0 c	3.1 c	3.2 bc	5.0 ab	6.5 a
Mg	-3.2 c	-1.6 ab	-1.4 ab	-2.0 b	-1.7 ab	-0.7 a
Ca	3.9 c	7.5 b	7.5 b	7.6 b	8.3 b	14.0 a
Al/Ca	1.1 a	0.9 b	1.0 ab	1.0 ab	1.1 a	1.0 ab
NH4/K	4.1 d	4.4 d	5.6 c	7.4 b	8.2 b	12.7 a
NH4/Mg	11.1 c	11.3 c	15.1 c	21.5 b	24.6 b	34.3 a
Heumen						
NO3	-3.7 a	-0.9 a	1.2 a	-1.9 a	0.3 a	0.6 a
NH4	32.8 a	28.7 a	21.1 b	-30.0 c	-39.6 c	-183.7 d
H	5.3 a	6.2 a	5.0 a	6.0 a	8.2 a	8.8 a
Al	5.5 e	6.1 de	6.9 cd	7.4 c	10.1 b	16.9 a
K	-5.3 d	-3.7 cd	-1.5 bc	-3.2 c	2.8 a b	3.6 a
Mg	-6.6 d	-5.8 c	-5.4 bc	-5.5 c	-4.4 ab	-4.0 a
Ca	-7.2 b	-6.9 ab	-5.0 ab	-6.3 ab	-5.0 ab	-4.6 a
Al/Ca	1.6 ab	1.8 ab	1.3 b	2.0 ab	2.0 ab	3.0 a
NH4/K	3.3 d	4.6 c	4.9 c	6.7 b	6.8 b	12.6 a
NH4/Mg	12.8 e	17.4 d	18.9 c	24.7 b	26.7 b	48.2 a
Budel						
NO3	54.8 c	68.7 c	165.4 b	183.5 b	233.2 b	355.6 a
NH4	-14.2 a	-45.5 b	-94.1 c	-145.1 d	-192.6 e	-482.8 f
H	6.5 c	6.7 c	9.6 bc	12.9 ab	15.1 ab	23.7 a
Al	5.8 e	7.6 e	16.1 d	23.9 c	37.4 b	100.8 a
K	-4.7 d	-0.1 c	3.5 b	3.7 b	7.7 ab	9.3 a
Mg	-3.3 c	-1.9 b	1.0 a	1.6 a	3.4 a	4.1 a
Ca	16.6 e	23.0 d	49.7 c	52.0 bc	63.1 ab	69.5 a
Al/Ca	.20 e	.23 de	.27 d	.38 c	.51 b	1.30 a
NH4/K	.20 b	.17 bc	.14 bc	.14 bc	.10 c	.58 a
NH4/Mg	.52 b	.39 bc	.26 c	.29 bc	.21 c	1.20 a
Orvelte						
NO3	52.0 a	134.1 a	25.0 a	66.8 a	12.3 a	4.7 a
NH4	52.8 a	36.2 a	14.2 b	-14.6 c	-32.4 c	-220.6 d
H	73.4 a	86.6 a	75.2 a	65.9 a	78.5 a	91.4 a
Al	3.8 b	5.6 b	6.3 b	5.2 a b	6.3 ab	8.0 a
K	8.3 b	13.4 ab	10.5 ab	10.7 a b	12.4 ab	17.1 a
Mg	-5.8 a	0.8 a	-3.8 a	-1.9a	-2.2 a	1.8 a
Ca	0.7 a	9.9 a	3.4 a	4.8 a	4.7 a	11.0 a
Al/Ca	0.5 a	0.3 a	0.5 a	0.4 a	0.4 a	0.4 a
NH4/K	1.8 d	2.0 d	3.6 c	4.3 c	5.1 b	7.3 a
NH4/Mg	9.2 b c	6.4 c	15.3 ab	16.5 ab	20.6 a	23.2 a

In the **Heumen** soil all variables, except net water, nitrate and H^+ fluxes, were significantly affected by the ammonium treatment and also varied with time (Table 2). Differences between the treatments in ammonium, aluminium and magnesium fluxes and the NH_4/Mg ratio during the experiment were not always similar. Again most net nutrient fluxes and the ammonium/cation ratios in the Heumen soil showed a clear gradient with the enhanced ammonium deposition (Table 3). Generally, those at the lowest load were significantly smaller than those at the highest ammonium load. The ammonium efflux was significantly smaller at the 56 kg treatment than at both lower loads and turned into accumulation at higher loads. The aluminium efflux increased and the amount of all basic cations accumulated in the columns decreased with the enhanced ammonium treatment. Only the potassium flux turned into an efflux at the 112 kg or higher load. Net nitrate fluxes clearly fluctuated with treatment and no obvious trend with time could be established (Figure 2). Net ammonium fluxes at all treatments initially increased and subsequently stabilized around zero. Though at the highest ammonium load ammonium accumulation decreased very fast the flux stabilized at -100 μmol. The Al/Ca ratio showed no clear trend with treatment nor with time though it appeared from Figure 2 that during the experiment this ratio was always highest at the highest load. Both ammonium/cation ratios showed similar trends and increased in time with treatment.

In the **Budel** soil all but water fluxes varied with the ammonium treatment (Table 2). Except for H^+ and aluminium these fluxes also varied with time. All net nutrient fluxes, except ammonium showed a clear raising gradient with the enhancing ammonium treatment (Table 3). The net ammonium flux showed an inverse relation and its accumulation increased with increasing deposition and hardly fluctuated with time (Figure 3). Nitrate left the columns at all treatments as did aluminium, H^+ and calcium. At the lowest loads (11.2 and 28 kg) magnesium and potassium did not leave the columns (Table 3). According to Table 3 all ratios showed a clear gradient with treatment though differences were very small. Figure 3 shows that at the highest loads (84, 112 and 280 kg) the Al/Ca ratio clearly rose and highest values were reached at the highest load. This also holds for the ammonium/cation ratios but no clear gradient with time could be observed.

Treatment effects in the **Orvelte** soil were less profound than in the other soil types. Only net ammonium, aluminium and potassium fluxes and the ammonium/cation ratios were affected by the ammonium treatment and they even not always varied with time (Table 2). In Table 3 is shown that ammonium left the column at the lower treatments and accumulated in the columns treated with 84, 112 and 280 kg ammonium. All other nutrients except magnesium showed effluxes at all treatments. Figure 4 shows that the net ammonium efflux decreased during the experiment at the lower loads and eventually resulted in ammonium accumulation. However, at the highest treatment ammonium accumulation decreased with time and at a load of 112 kg the ammonium accumulation fluctuated. Nitrate effluxes and Al/Ca ratio did not show any clear gradient neither with treatment nor with time. Especially at the lowest treatment net nitrate and aluminium fluxes displayed a strange course in time. Both ammonium/cation ratios decreased at a load of 11.2 and 28 kg and clearly rose at the 112 and 280 kg NH_4-N. At both other loads no clear raising or decreasing trend could be observed.

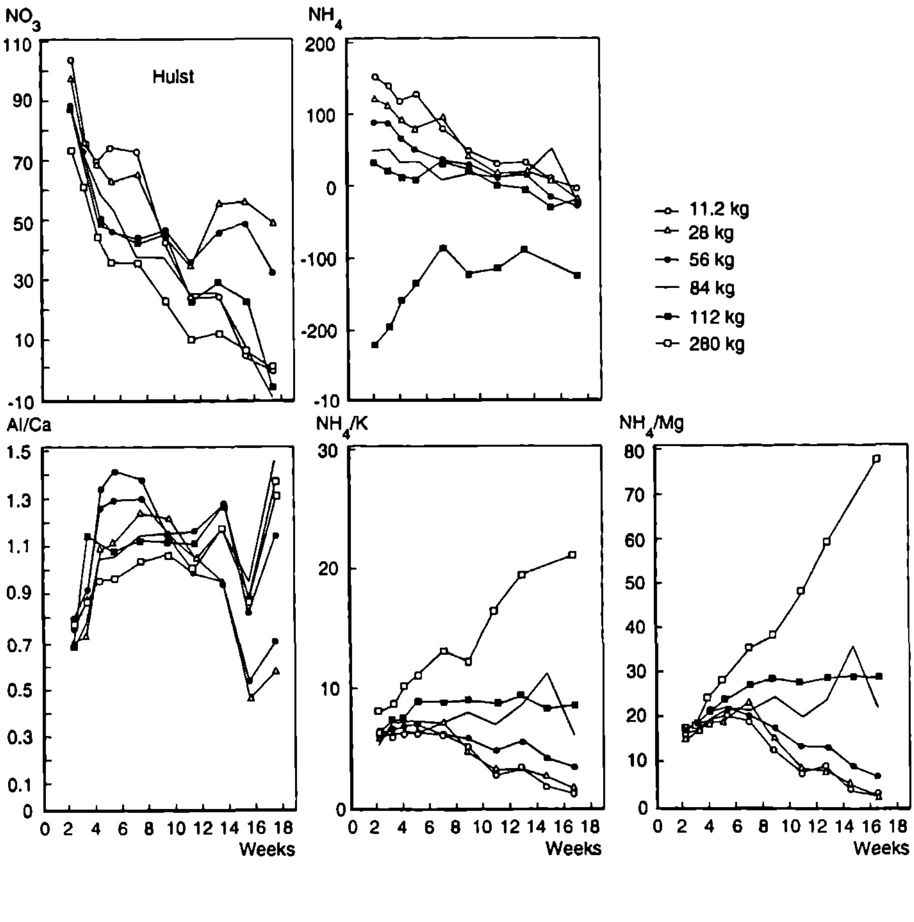

Figure 1: Weekly net nitrate and ammonium fluxes and Al/Ca, NH_4/K and NH_4/Mg ratios in percolation water of the Hulst soil per ammonium treatment.

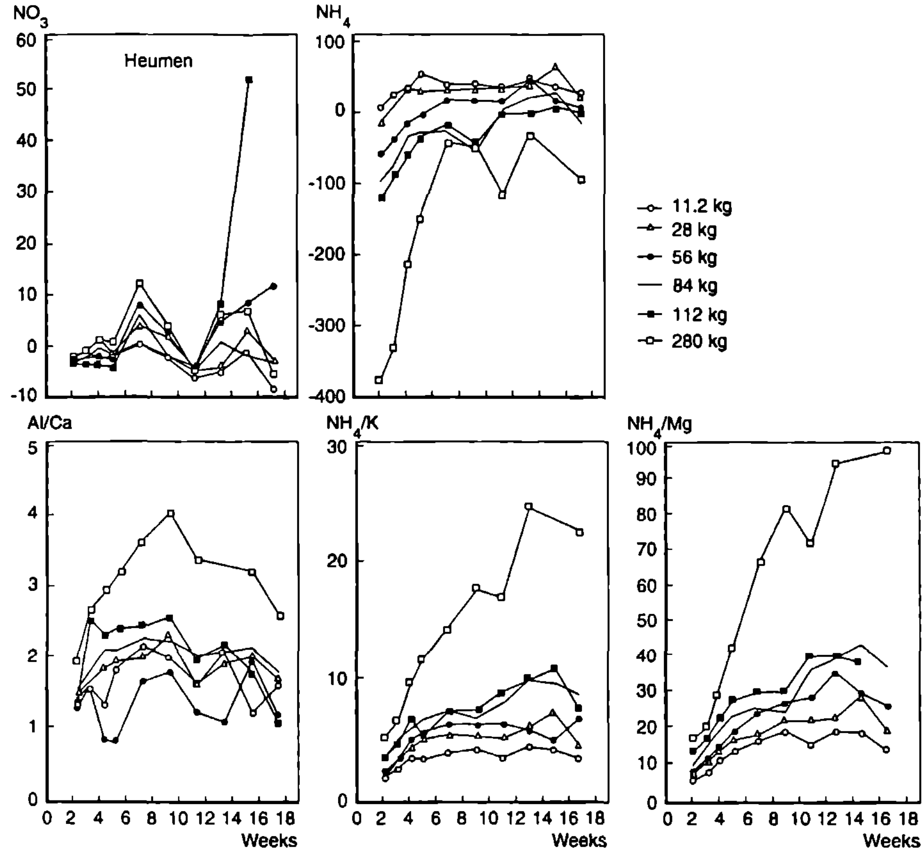

Figure 2: Weekly net nitrate and ammonium fluxes and Al/Ca, NH_4/K and NH_4/Mg ratios in percolation water of the Heumen soil per ammonium treatment.

Experiment II

In contrast to experiment I the presented fluxes of experiment II are all total (sum of half a year) net fluxes and no average weekly net fluxes are shown. ANOVA showed that there were no significant interactions between the three experimental variables. Furthermore, it demonstrated that all dependent variables except the NH_4/K ratio were affected by soil type (Table 4). Only ammonium fluxes and the ammonium/cation ratios were affected by the treatment and nitrate and the ammonium cation ratios were affected by the presence or absence of the litter layer (data not shown).

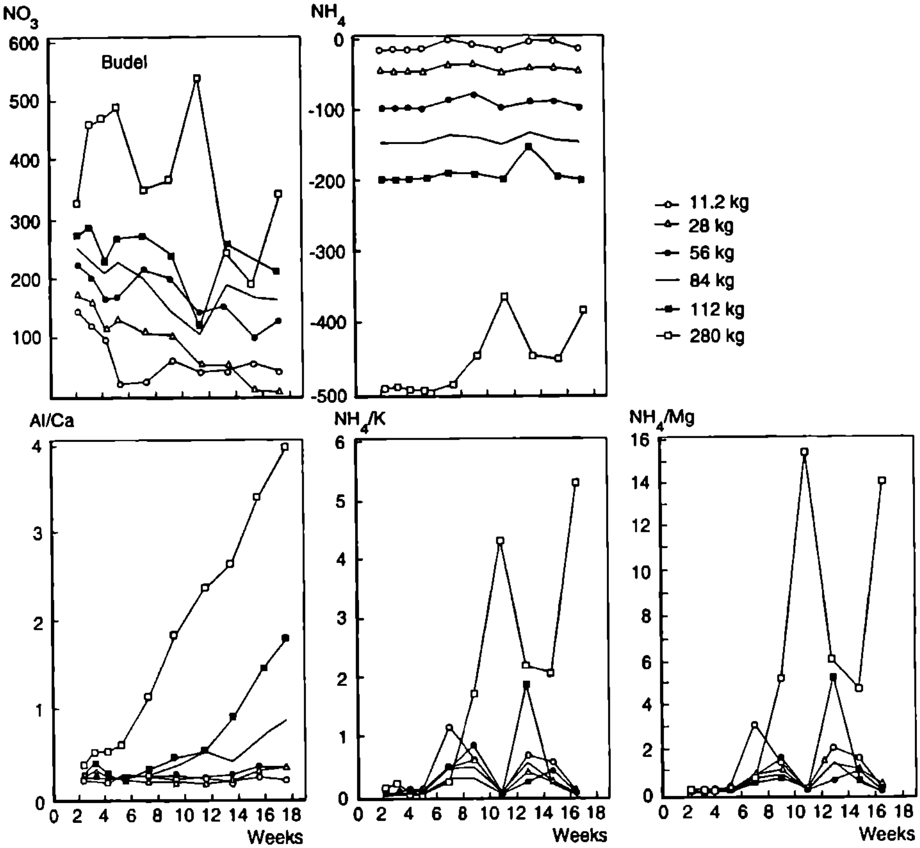

Figure 3: Weekly net nitrate and ammonium fluxes and Al/Ca, NH_4/K and NH_4/Mg ratios in percolation water of the Budel soil per ammonium treatment.

Table 5 reveals how average ratios and net nutrient fluxes per soil type were affected by ammonium treatment or litter layer. In this experiment both soil types were affected by the ammonium treatment in a similar way as in the first experiment. However, the differences between treatments were smaller, probably due to the lower deposition rate and smaller treatment gradient. Significantly more nitrate left the column with litter layer on the Heumen soil whereas this difference was almost significant ($P<0.1$) in the Hulst soil. NH_4/K and NH_4/Mg were significantly and almost significantly lower in the Heumen soil with a litter layer. In the Hulst soil with litter layer the NH_4/Mg ratio was significantly smaller.

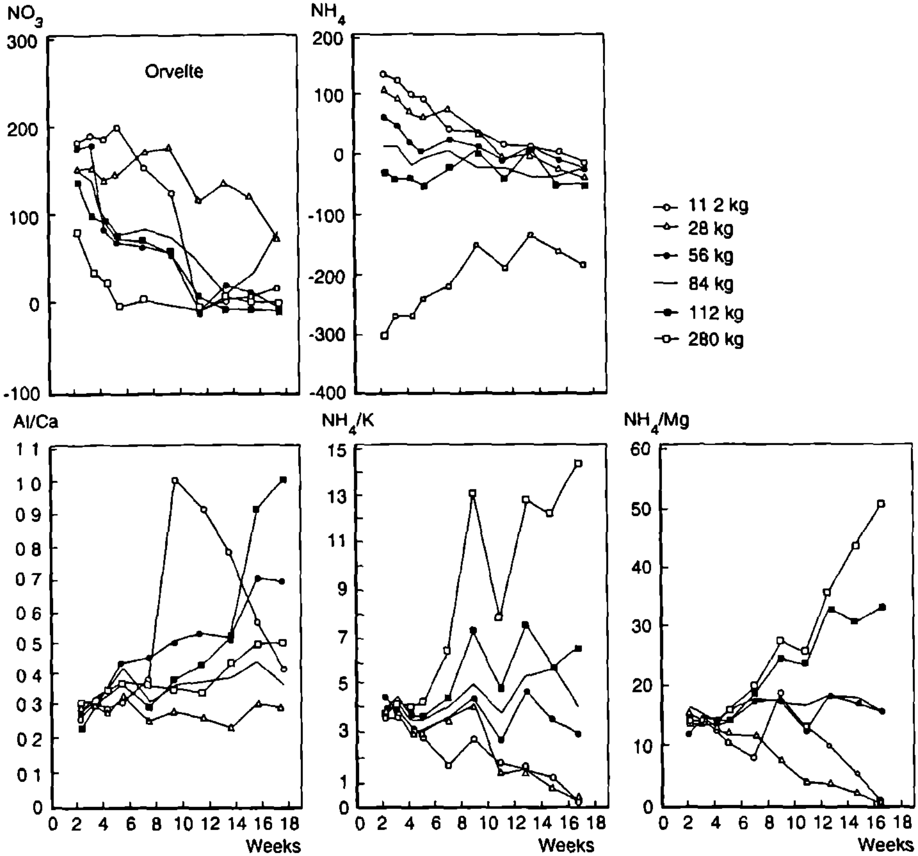

Figure 4: Weekly net nitrate and ammonium fluxes and Al/Ca, NH_4/K and NH_4/Mg ratios in percolation water of the Orvelte soil per ammonium treatment.

DISCUSSION

The present study clearly shows that ammonium deposition affects the mineral balance of the forest soils. In all soil types ammonium/cation or Al/Ca ratios increase with the enhanced ammonium deposition. However, the size of the ammonium flux onto the soil at which the mineral balance is adversely disturbed (c.q. threshold values of Ulrich (1983), Roelofs *et al.* (1985) and Van Breemen & Verstraten (1990) are exceeded) varied widely between the soil types. It appears from the Figures 1 to 4 that these critical ammonium fluxes can be roughly estimated between 28 and 56 kg for the Heumen soil, between 56 and 84 for the Hulst soil and between 84 and 112 kg NH_4-N.ha.y for the Orvelte and Budel soil respectively. In all soil types exceedance of the threshold value of the NH_4/K ratio in the percolation water accounted for the critical ammonium deposition. Only in the Budel soil high Al/Ca ratios caused the

Table 4: Average total net fluxes (µmol) and ratios after one half year of treatment per soil type (n=12). Average values are geometrical means. Different letters within a row indicate statistical differences, at a 5 % level according to Tukey's multiple-comparison procedure. Positive fluxes indicate efflux from the column and negative fluxes indicate accumulation or conversion in the columns.

	Heumen	Hulst
ml	-565 b	-723 a
NO3	1 b	1392 a
NH4	-619 b	276 a
H	73 b	219 a
Al	177 b	473 a
K	-135 b	62 a
Mg	-77 b	79 a
Ca	-80 b	126 a
Al/Ca	4.0 a	2.2 b
NH4/K	6.2 a	5.6 a
NH4/Mg	16.8 a	8.7 b

Table 5: Average ratios and total net fluxes (µmol) after one half year per ammonium treatment (n=4) and litter type (n=6) for each soil type. Different letters within a row indicate statistical differences, at a 5 % level according to Tukey's multiple-comparison procedure. Positive fluxes indicate efflux from the column and negative fluxes indicate accumulation or conversion in the columns.

	ammonium treatment			litter layer	
	28	56	112	present	absent
Hulst					
NO3	1625 a	1200 a	1381 a	1893 a	1017 a
NH4	958 a	304 a	-246 a	173 a	384 a
H	219 a	207 a	232 a	211 a	227 a
Al	504 a	519 a	406 a	463 a	484 a
K	28 b	45 b	123 a	51 a	74 a
Mg	95 a	48 a	97 a	83 a	75 a
Ca	145 a	87 a	152 a	147 a	106 a
Al/Ca	2.1 b	2.9 a	1.7 b	2.0 a	2.3 a
NH4/K	5.6 a	5.1 a	6.0 a	5.1 a	6.0 a
NH4/Mg	6.8 b	9.0 ab	10.6 a	6.8 b	11.0 a
Heumen					
NO3	-29 a	27 a	11 a	154 a	-58 b
NH4	38 a	-237 ab	-1181 b	-681 a	-552 a
H	58 b	66 ab	102 a	69 a	78 a
Al	127 a	165 a	266 a	176 a	178 a
K	-163 a	-121 a	-85 a	-92 a	-158 a
Mg	-88 a	-69 a	-67 a	-71 a	-82 a
Ca	-93 a	-75 a	-62 a	-78 a	-83 a
Al/Ca	4.2 a	3.7 a	4.1 a	3.7 a	4.3 a
NH4/K	4.6 b	6.0 ab	8.4 a	5.0 b	7.6 a
NH4/Mg	13.4 b	14.3 b	24.6 a	14.6 a	19.4 a

disturbed mineral balance.

From the estimated mean net nitrate fluxes (Table 1) nitrification rates in the Heumen, Hulst, Orvelte and Budel soil can be calculated at 0, 3.9, 4.4 and 17.7 kmol $NO_3.ha^{-1}.yr^{-1}$ respectively. In the Budel soil ammonium accumulation and concomittantly high ammonium/cation ratios were obviously prevented by the fast conversion of ammonium to nitrate whereas on the other hand proton production as a result of the high nitrification rate eventually lead to high Al/Ca ratios. The relatively high calcium content of the soil, which clearly buffered part of the H^+ percolating through the column, prevented the Al/Ca ratio from reaching the threshold value at lower ammonium deposition as aluminium effluxes increased considerably with increasing deposition. Only at the highest deposition also NH_4/K reached threshold values when nitrification rates were too low to prevent ammonium accumulation. Generally, the nitrification rate in the soil is regulated by ammonium availability, pH, allelopathy, moisture and temperature (Killham, 1990). In this soil the low pH in the column treated with the highest deposition probably accounted for the incomplete nitrification. Since the nitrification rate increased with the enhanced deposition, substrate inhibition (Focht & Verstraete, 1977) does not seem to be responsible for the incomplete nitrification and similar moisture and optimal temperature conditions were prevailing in all soil columns at all treatments.

In all other soil types nitrification rates were lower and did not increase with the enhancing deposition but seem to have an optimum. These lower nitrification rates may be due to the lower pH (H_2O) values initially measured in these soils (3.7, 3.9 and 4.2 in the Hulst, Heumen and Orvelte soil respectively and 4.4 in the Budel soil). However, many investigators have proved that nitrification also takes place in acid soils (Kriebitzsch, 1979; De Boer *et al.*, 1988; Killham, 1990; Stams & Marnette, 1990; Tietema *et al.*, 1992). Nitrification rates can also be inhibited by phenolic or other toxic compounds as these afforested soils were formerly covered by heathlands (Kriebitzsch, 1978; Kinzel, 1982). The soil from Budel used to be agricultural land before forests were planted. Furthermore unbenificial moisture conditions in the columns especially at the bottom of the columns, may lead to water saturation, concomitantly to oxygen deficiency and prevent nitrification (Beck, 1979) or may lead to denitrification (Tietema & Verstraten, 1991). However, percolation columns equipped with porous plates to create a constant water flow and to prevent water saturation, revealed similar nitrate levels as soil columns without a plate. This comparitive study showed that in columns containing mineral soils without loam, nitrification rates are not affected by water accumulation (data not shown).

In the Heumen soil the lowest nitrification rate was measured and the threshold value for the NH_4/K was already exceeded at an ammonium deposition between 28 and 56 kg NH_4-N. In fact this was the only soil type in which the NH_4/K increased at all treatments after the start of the experiment (Figure 1). This could indicate that the

NH_4 fluxes administered to the columns were higher than under natural conditions. However, in the field much higher deposition fluxes (70 $kg.N.ha^{-1}.yr^{-1}$ according to Houdijk & Roelofs, 1991) were found. The absence of ammonium uptake by the vegetation in soil columns may account for this difference but does not explain why in both other low nitrifying soil types this ratio showed no initial increase. From the comparison of the chemical soil composition it appeared that the exchangeable ammonium amount in the Heumen soil was much lower than in both other soil types which were probably already ammonium saturated. The relatively young Heumen forest seems to be able to take up more ammonium than the other forests. However, as soon as this forest will be saturated ammonium will accumulate at the present deposition. Then deposition indeed has to decrease below 28-56 kg N to restore the mineral balance.

In the field situation at a deposition of 130 kg NH_4-$N.ha^{-1}.yr^{-1}$ (Houdijk & Roelofs (1991) the NH_4/K ratio in the Hulst soil was larger than 5. Removal of the vegetation had no effect on this ratio probably due to the low ammonium uptake of the trees compared to the input. The percolation experiment clearly showed that restoration of the mineral balance is possible when the ammonium deposition is lowered to 56 kg NH_4-N. In the Orvelte soil the NH_4/K ratio has not yet exceeded the critical value of 5 at a field deposition of 70 kg NH_4-$N.ha^{-1}.yr^{-1}$ (Houdijk & Roelofs, 1991). Higher deposition will immediately lead to exceedance of the threshold value while lowering of the deposition will lead to a clear decrease of the ratio.

Microclimatic conditions for the nitrification process are considered to be better in the litter layer covering the mineral soil than in the mineral soil itself. According to Tietema *et al.* (1992) in Dutch coniferous forest soils nitrification is mainly located in the ecto-organic soil layer whereas Vonk (1988) found that most nitrification takes place in the mineral soil. Comparison of nitrification rates in soil columns with and without litter layer indeed showed that average nitrification rates were higher in columns with a litter layer than without this layer (8.6 and 0.7 kmol $NO_3.ha^{-1}.yr^{-1}$ compared to 4.6 and 0 kmol $NO_3.ha^{-1}.yr^{-1}$ in the Hulst and Heumen soil respectively). Generally, also the mineral soil balance seems to be more favourable in the presence of litter. This may lead to some overestimation of the critical ammonium load for these soils. However, the presence of litter on soil columns does not change low nitrifying soils into soils with high nitrification rates.

In conclusion: This study showed that, despite the absence of vegetation, soil percolation experiments can be used to predict the effects of ammonium deposition on soils of nitrogen saturated forests. The mineral balance in soil columns infiltrated with an ammonium containing solution is similar to that of the forest soils in the field with the same deposition levels. A change in the ammonium treatment compared to the field deposition leads to a comparable change in the level of disturbance of the mineral balance respectively. The threshold value of the NH_4/K ratio in low nitrifying soils is already exceeded at a much lower ammonium deposition than in soils with higher nitrification rates. However in low to moderately nitifying soils the contribution of nitrification in the litter layer may have some effect on the critical deposition level measured by use of litter free soil columns. High nitrifying soils have the highest critical deposition level and in these soils the aluminium/calcium rather than the ammonium/potassium ratio seems to be responsible for the disturbance of the mineral balance in the soil. In low nitrifying soils as well as in high nitrifying soils leaching of base cations increases with increasing ammonium deposition.

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CHAPTER 7

DISTRIBUTION AND DECLINE OF ENDANGERED HERBACEOUS HEATHLAND SPECIES IN RELATION TO THE CHEMICAL COMPOSITION OF THE SOIL

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ABSTRACT

High atmospheric deposition of ammonium affects the physical and chemical status of the soil, increasing nitrogen availability, soil acidity and the mobilization of toxic metal ions. To investigate whether and how the decline of several herbaceous plant species in Dutch heathlands is associated with these processes, the chemical composition of the soil on which these species grow has been compared with the soil on which heathland species such as *Calluna vulgaris* (L.) Hull, *Erica tetralix* L. and *Molinea caerulea* (L.) Moench dominate.

The discrimination between both soil types was primarily based on differences in pH (H_2O), pH (NaCl) and the aluminium/calcium ratio in the waterextracts. Within the group of endangered herbaceous heathland species these soil parameters also varied. This led to a division into 4 groups of species:

- Dominating species growing on acid soils
- Herbaceous species growing together with dominating species on acid soils
- Herbaceous species growing together with dominating species on moderately acid soils
- Herbaceous species growing together with dominating species on weakly acid soils

This study indicated that, unlike the decline of heather species, the decline of herbaceous species is not likely to be due to increased competition from grass species as a result of eutrophication. Soil acidification and the changed mineral balance in the soil are most likely to be responsible for the decline of all three groups of herbaceous plant species.

INTRODUCTION

Until the beginning of this century heathlands covered 800,000 ha of the pleistocene soils in the Netherlands. Since then the area of heathland decreased dramatically to 40,000 ha in 1980. The initial decline was mainly due to land reclamation for agricultural use. However, the decline during the last decades can be ascribed mainly to changes in management and atmospheric deposition. Since 1950 53 % of all plant species in the Netherlands, and in heath- and chalk-grasslands even 86 %, showed some sort of decline (Centraal bureau voor Statistiek, 1983) and dramatic shifts in species composition could be observed (Bobbink and Willems, 1987; Heil and Diemont, 1983)

In dry heathlands the originally dominant dwarfshrub *Calluna vulgaris* (L.) Hull has been largely replaced by the grasses *Molinia caerulea* (L.) Moench and *Deschampsia flexuosa* (L.) Trin.. In wet heathlands *Molinia* nowadays dominates *Erica tetralix*. Generally, the raised nutrient availability in the soil, due to the extensified management practices and increased inputs of air-borne nutrients, is accepted to have caused this change (Aerts, 1989; Berendse and Aerts, 1987; Grime, 1979; Heil and Diemont, 1983; Heil *et al.*, 1988).

According to Van der Eerden *et al.*. (1990) other heathland species, particularly those of the Violion caninae alliance, are probably even more sensitive to atmospheric deposition than heather species. However, unlike the latter the former seem to disappear already before grasses become dominant. At present many herbaceous plants have become rare and their occurrence is restricted nowadays to small patches on very few locations.

In the Netherlands primarily sulphuric and nitrogenous compounds, in particular ammonium, contribute to atmospheric deposition (Houdijk and Roelofs, 1991). Atmospheric deposition is known to affect the chemical and physical status of the soil, increasing nutrient availability, soil acidity and the mobilization of toxic metal ions (Houdijk *et al.*, 1993; Van Breemen *et al.*, 1982). However until now it is not known which of these soil processes are likely to attribute to the decline of the threatened herbaceous heathland species. For preservation of the species diversity in heathland ecosystems knowledge about these processes is essential. In the present study soil characteristics of the few remaining localities of several rare herbaceous species and those of *Molinia, Erica* and *Calluna* vegetations were used to establish whether direct and/or indirect effects of acidification and/or eutrophication resulting from atmospheric deposition may be responsible for this decline.

MATERIALS AND METHODS

Site selection

All investigated species were found in heathlands on the loam-free, mineral soils in the Southern, Central and Eastern parts of the Netherlands as well as in the dunes in the Western parts. The selection of the endangered herbaceous heathland species (from now on referred to as herbaceous species) was primarily based on the availability of sufficient localities (ca. 10 per species). Furthermore, the abundance and the distribution of the selected species should have declined recently (Mennema *et al.*, 1985; Van De Meijden *et al.*, 1983). *Molinia* (referred to as grass species), *Calluna* and *Erica* (referred to as heather species) locations were selected on the absence of herbaceous species. These sites were dominated by either of the plant species, covering at least 60 %. Table 1 shows which plant species were studied and the number of sites sampled.

Table 1: The investigated heathland species, the alliances they belong to (Vc:Violion caninae, Gc:Calluno Genistion pilosae, E:Ericion tetralix, TA:Thero-Airion), their division into groups and the number of sites sampled.

Species	Alliance	Number of localities
Calluna vulgaris (L.) Hull	Gc	10
Erica tetralix L.	E	9
Molinia caerulea (L.) Moench		10
Lycopodium clavatum L.	E	13
Rhynchospora fusca (L.) Ait.f.	E	12
Arnica montana L.	Vc	9
Genista pilosa L.	Gc	9
Gentiana pneumonanthe L.	Vc	11
Lycopodium inundatum L.	E	12
Narthecium ossifragum (L.) Huds.	E	10
Dactylorhiza maculata (L.) Soo	Vc	10
Genista tinctoria L.	Gc	10
Pedicularis sylvatica L.	Vc	13
Polygala serpyllifolia Hose	Vc	10
Thymus serpyllum L.	TA	10

Sampling

Sampling took place in May, June and July in random order. At each site, between or close to the roots of the selected species soil samples were taken, consisting of four subsamples of mineral sandy soil, taken just below the litter layer to a depth of 10 cm, using a brass tube (length 10 cm, ø 3 cm). The subsamples were put together in a polyethylene bag and mixed. 70 g of fresh soil were put into a 500 mL polyethylene bottle together with 200 mL bidistilled water or a 0.2 *M* NaCl solution and shaken for 1 h, after which pH was measured. After centrifugation for 15 min at 275,000 g, the supernatant was stored at -28 °C until analysis. Dry soil was combusted for 4 h at 550 °C in order to estimate the organic matter content.

Ca, Mg, Al, Mn, Zn, Fe, S and P were measured with an Inductively Coupled Plasma spectrophotometer (ICP), type IL Plasma 200. K and Na were asessed using a Technicon Flame photometer IV. Colorimetrical determination with a Technicon AAII system according to Technicon Methodology (Technicon corporation, 1969) and Kempers and Zweers (1986) was used for NO_3 and NH_4 respectively, and a Technicon AAI-system according to Technicon Methodology (Technicon corporation, 1969) and O'Brien (1962) for Cl.

Statistics

Scheffé's multiple comparison procedure (performed with the General Linear Models (GLM) procedure) was used as a test of significance for differences between means (SAS Institute Inc., 1985). This statistical operation was performed on log-transformed data, since these fitted better to the conditions of normality (Sokal and Rohlf, 1981). For presentation, the data were backtransformed. Consequently, the mean values given are geometrical means, which are the maximum likelihood estimators of the population medians.

RESULTS

GLM analysis of individual soil variables of groups of species are presented in Table 2. Highest values of pH (H_2O), pH (NaCl) and waterextractable calcium concentrations and lowest Al/Ca ratios were measured in the soils of herbaceous species. Soils of *Molinia* vegetations showed the highest levels of waterextractable nitrate, ammonium and phosphorus and salt-extractable ammonium. Waterextractable potassium levels and the ammonium/nitrate ratios in the soils of herbaceous species were of intermediate value.

Table 2: Geometrical means of organic matter (% DW), water and saltextractable variables (μmol. kg^{-1} DW) and ratios ($mol.mol^{-1}$) of herbaceous, grass and heather species. Different letters within a row indicate statistical differences between the soil variables of the three groups of heathland species at the 5 % level according to scheffé's multiple comparison analysis.

	Herbaceous species	Grass species	Heather species
org.matter	5.3 b	10.6 a	7.0 ab
Waterextractable variables			
NO3	18 b	71 a	17 b
P	7 b	28 a	13 b
pH	4.6 a	3.9 b	4.2 b
NH4	74 b	152 a	89 b
K	108 ab	175 a	61 a
Mg	22 a	17 a	10 a
Ca	84 a	13 b	7 c
Al	220 a	228 a	158 a
Al/Ca	2.6 b	17.4 a	23.5 a
NH4/NO3	4.1 ab	2.2 b	5.1 a
Salt extractable variables			
pH	3.8 a	2.9 b	3.0 b
NH4	97 b	367 a	194 b
K	240 b	453 a	272 ab
Mg	276 a	342 a	312 a
Ca	1432 a	1251 a	912 a
Al	903 a	2238 a	1725 a

Table 3. Geometrical means of organic matter (% DW), several water and saltextractable soil variables (in $\mu mol.\ kg^{-1}$ DW) and ratios (in $mol.mol^{-1}$) of heathland species. $N/N=NH_4/NO_3$.

group	species	Waterextractable variables										Saltextractable variables					
		pH	NH4	NO3	P	K	Mg	Ca	Al	Al/Ca	N/N	pH	NH4	K	Mg	Ca	Al
1	*C. vulgaris*	4.1	78	17	12	53	8	6	137	25.2	4.6	3.0	241	282	300	1071	1779
1	*E. tetralix*	4.2	98	12	14	68	12	10	174	17.8	8.0	3.0	147	281	359	925	1733
1	*M. caerulea*	3.9	152	71	28	119	17	13	228	17.4	2.2	2.9	367	453	342	1251	2238
2	*L. clavatum*	4.1	39	20	5	54	7	30	176	5.9	2.0	3.3	59	93	149	454	2485
2	*R. fusca*	4.2	112	22	8	175	11	48	259	5.4	5.0	3.5	118	292	126	542	2648
3	*A. montana*	4.5	86	13	6	114	12	55	246	4.5	6.4	3.6	104	273	241	963	2703
3	*G. pilosa*	4.5	84	12	3	118	11	37	136	3.7	7.1	3.7	112	184	113	377	983
3	*G. pneumonanthe*	4.4	140	34	15	166	31	109	392	3.6	4.2	3.5	123	397	337	1917	2123
3	*L. inundatum*	4.7	47	10	3	98	15	58	128	2.2	4.8	4.0	60	153	52	290	856
3	*N. ossifragum*	4.4	147	26	9	318	56	87	222	2.5	5.6	3.6	150	487	451	994	1247
4	*D. maculata*	4.8	107	35	12	74	51	242	348	1.4	3.1	3.8	110	194	1043	7023	394
4	*G. tinctoria*	5.4	37	14	11	86	43	126	134	1.1	2.6	4.3	58	294	345	4519	133
4	*P. sylvatica*	4.9	61	15	6	104	33	151	285	1.9	4.0	4.0	117	220	497	3870	510
4	*P. serpyllifolia*	4.6	81	17	7	90	25	134	246	1.8	4.8	3.7	105	297	397	1239	942
4	*T. serpyllum*	5.2	53	15	13	72	40	154	209	1.4	3.5	4.3	111	320	860	5179	217

However, individual soil variables of herbaceous species showed large variations (Table 3). Based on the most discriminating soil variables derived from table 2 (pH, NH_4, NO_3, Ca, Al/Ca and to a lesser extent NH_4/NO_3) and considering the individual values of these soil variables the twelve herbaceous species are divided into three groups of species and compared with the group of dominant heathland species (*Molinia*, *Erica* and *Calluna*)(Table 4).

Table 4. Geometrical means of organic matter (% DW), water and saltextractable variables (µmol. kg^{-1} DW) and ratios ($mol.mol^{-1}$) of group 1, 2, 3 and 4. Different letters within a row indicate statistical differences between these four groups of species at the 5% level according to scheffé's multiple comparison analysis. For explanation of group division see Table 3.

	Group1	Group2	Group3	Group4
organic matter	8.1 a	5.8 ab	5.5 ab	4.8 b
Waterextractable variables				
NO3	30 a	21 a	17 a	18 a
PO4	18 a	6 b	6 b	9 b
pH	4.1 c	4.1 c	4.5 b	5.0 a
NH4	118 a	64 a	93 a	63 a
K	83 a	95 a	148 a	85 a
Mg	13 bc	9 c	21 ab	37 a
Ca	10 c	37 b	66 b	157 a
Al	189 a	222 a	208 a	235 a
Al/Ca	19.7 a	5.7 b	3.2 b	1.5 c
NH4/NO3	3.9 ab	3.1 b	5.4 a	3.5 ab
Saltextractable variables				
pH	3.0 c	3.4 b	3.7 a	4.0 a
NH4	269 b	821 a	1040 a	982 a
K	347 a	157 b	273 ab	259 ab
Mg	342 ab	137 c	183 bc	567 a
Ca	1074 b	494 c	731 bc	4461 a
Al	1923 a	2562 a	1421 a	360 b

The first group of species consists of the heather species and *Molinia*. pH in the soils of this group was low (3.9-4.1) and the lowest waterextractable calcium levels and the highest Al/Ca ratios were measured. NH_4/NO_3 ratios in these soils were of moderate value. *Lycopodium clavatum* L. and *Rhynchospora fusca* (L.) Ait. f. form the second group of species growing on soils with similar pH values as those in the soil of the dominant species. However, the Al/Ca ratio in the soil of group 2 was lower. The remaining herbaceous species all grow on soils with higher pH values. The third group consists of five species: *Arnica montana* L., *Genista pilosa* L., *Gentiana pneumonanthe* L., *Lycopodium inundatum* L. and *Narthecium ossifragum* (L.) Huds.. The pH and the NH_4/NO_3 ratio in the soil of this group were higher than and the Al/Ca ratio and waterextractable calcium levels were comparable to those of group 2. Group 4 was formed by *Dactylorhiza maculata* (L.) Soo, *Genista tinctoria* L.. *Pedicularis sylvatica* L., *Polygala serpyllifolia* Hose and *Thymus serpyllum* L.. In the soils of this group highest pH values, water and saltextractable calcium levels and lowest saltextractable aluminium levels and Al/Ca ratios were measured.

DISCUSSION

Soil composition

At present Dutch heathlands are dominated by grass species like *Molinia* and *Deschampsia* and the dwarfshrub species *Calluna* and *Erica*. This study confirms the findings of Roelofs *et al.* (1985) and De Boer (1990) that both heather and grass species prefer acid soils whereas the soil composition merely differs in nutrient, particularly nitrogen, content: soils of heather species have low and those of grass species have high nitrogen contents. The enhanced nutrient availability, primarily resulting from the high ammonium deposition and the reduced nutrient removal due to extensified management, was found to account for the observed changeover from dwarfshrub to grass dominated heathlands (Aerts, 1989; Heil and Diemont, 1983; Roelofs, 1986).

The comparison on basis of individual soil variables emphasized the importance of the ratios of aluminium to calcium besides pH and calcium (Table 3). Generally, nutrient levels (nitrogen and phosphorus) in the soils of the herbaceous species were much lower than in the soil of *Molinia* and comparable with those in the soils of dwarfshrub species. These findings, together with the fact that herbaceous species are not specifically replaced by grass species but that they are also replaced by heather species, indicate that acidification probably forms the greatest threat for their exsistence. Van Dam *et al.* (1986) also correlated the decline of herbaceous species with the effects of acid rain due to the deposition of acid and acidifying substances as ammonium, sulphate and nitrate and Fennema (1990) attributed the decline of *Arnica montana* to the acidifying effect of the enhanced ammonium deposition rather then to its eutrophicating effect.

Soil acidification

The acidifying effect of atmospheric deposition can either be direct or indirect. Although all the investigated herbaceous species show a preference for acid soils (Ellenberg, 1979; Westhoff and Den Held, 1969), the pH (H_2O) of these soils is generally somewhat higher than in the soil of the dominating species. Indirectly, soil acidification might lead to the loss of exchangeable base cations and the mobilization of toxic metal ions like aluminium (Ulrich, 1983). In the present study however, aluminium content in soils of dominating and herbaceous species did not differ significantly. Kroeze *et al.* (1989), who tested aluminium susceptibility of several species of the Violion caninae alliance, concluded that the decline was not likely to be due to an increased aluminium availability. Generally, organically complexed aluminium is assumed to be less toxic than inorganic aluminium forms. However, Mulder (1988) found that in the mineral layer of Dutch heathland soils 95% of aluminium is available in as aquous Al^{3+}. Aluminium toxicity may also be reduced by calcium and magnesium and several investigators (Boxman *et al.*, 1991; Ulrich, 1983) found that the concentration of calcium relative to aluminium formed a better measure to estimate aluminium toxicity. This Al/Ca ratio appeared to be much higher in the soil of the dominating species (Table 3). Further acidification of the soil of herbaceous species may lead to higher Al/Ca ratios which may in turn cause the decline of these species.

Generally, it is assumed that nitrification is limited in acid heathland soils. In heathland soils covered with a healthy dwarfshrub vegetation nitrification rates seem to be inhibited in soils with a pH of 4.0-4.2 (Roelofs *et al.*, 1985). However, in grass dominated heathland soils with similar pH, nitrate production is relatively high (De Boer, 1990). In the present study nitrification in the soil of the grass species is reflected by the low NH_4/NO_3 ratios but even more clearly by the high nitrate contents of these soils. Nitrate contents, in both dwarfshrub dominated heathland soils and in the soils of the herbaceous species, were lower. According to Kinzel (1982) *Ericacae* show very low nitrate reductase activity which may indicate that these species hardly use nitrate to meet their N-demand. In contrast, some current experiments with several herbaceous species showed that they generally seem to prefer nitrate when both nitrogen-forms are offered and that cation uptake benefits from nitrate-nutrition (per. com. De Graaf). When this is the case it can explain the relatively high NH_4/NO_3 ratios in the soils of herbaceous species. Further acidification might slow down nitrate production and may prevent nitrate nutrition which accounts for the decline of the investigated herbaceous species.

Discrimination of groups of species

Processes that may cause the decline of herbaceous heathland species are discussed by comparing the chemical soil composition of three groups of herbaceous species with that of the dominating heathland species (*Molinia*, *Calluna* and *Erica*) growing on acid soils with high Al/Ca ratios.

Soils of species of group 2 (*Rhynchospora fusca* and *Lycopodium clavatum*) have similar pH values but lower Al/Ca ratios. Therefore, the species belonging to group 2 may decline as a result of an indirect effect of acidification, i.e. raised Al/Ca ratios.

The relatively high pH (4.5-4.8) values in the soil of group 4 (*Dactylorhyza maculata, Polygala serpyllifolia, Pedicularis sylvatica, Thymus serpyllum* and *Genista tinctoria*) together with the high base cation contents indicate that this group of species occurs on weakly buffered soils in the cation exchange buffer range (pH 4.2-5.0) (Ulrich, 1983).

Group 3 (*Lycopodium inundatum, Gentiana pneumonanthe, Arnica montana, Narthecium ossifragum* and *Genista pilosa*) forms an intermediate group. These species grow on soils with higher pH values than in the soil of group 1 and 2 but pH values are lower than those in soils of group 4. Calcium levels and Al/Ca ratios are similar to those of group 2 but lower and higher than in group 4 respectively. Soil acidification probably forms the greatest threat for both groups of species (group 3 and 4). Eventually, however, also increased Al/Ca ratios and decreased NH_4/NO_3 ratios in the soil as a consequence of soil acidification may also cause the decline.

Conclusions

Generally the soils of the threatened herbaceous heathland species range from acid to weakly acid and are nutrient-poor. Largest discrimination between these soils and those of the dominant heathland species (*Calluna, Erica* and *Molinia*) was related to pH, the Al/Ca ratio and to a lesser extent to the NH_4/NO_3 ratio. On account of the soil composition the heathland species can be divided into four groups. This study suggests that the direct and indirect effects of acidification, due to enhanced atmospheric deposition, may be responsible for the decline of all three groups of herbaceous species.

In order to provide causal relationships ecophysiological and culture experiments were carried out with some of the species involved. The results of these experiments will be discussed in a next paper.

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CHAPTER 8

EFFECTS OF PH, ALUMINIUM AND AMMONIUM ON THE SURVIVAL AND NUTRIENT SUPPLY OF *THYMUS SERPYLLUM*

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ABSTRACT

In the Netherlands many declining heathland species grow on soils which differ distinctly in pH from those of dominating heathland species like *Molinia caerulea* (L.) Moench, *Erica tetralix* L. and *Calluna vulgaris* (L.) Hull.

In this study experiments were conducted with *Thymus serpyllum* L. to investigate whether the decline of this species is related to the pH decrease, to increased aluminium concentrations or to changed NH_4/NO_3 ratios in the soil as a result of the enhanced ammonium deposition.

Pot experiments showed that *Thymus* was able to survive on soils with very low pH and high aluminium concentrations but that a disbalance between NH_4 and NO_3 in the soil solution resulted in a severe decrease of the vitality or even in death of *Thymus*. Hydroculture experiments confirmed that cation uptake of *Thymus* was adversely affected by the presence of NH_4. *Thymus* was able to use both nitrogen forms but NH_4 inhibited K, Mg and Ca uptake while NO_3 clearly stimulated K uptake. In contrast to *Thymus*, *Calluna* on hydroculture only used NH_4.

INTRODUCTION

The dramatic shift in species composition in Dutch heatlands is found to be related to the enhanced atmospheric deposition, in particular the ammonium deposition, of the last decades. The increased nutrient availability in the soil is generally accepted to account for the competition between the fast growing grass species and heather species with low growth rates (Roelofs, 1986; Berendse & Aerts, 1987; Heil *et al.*, 1988). However, the initial decline of the rare heathland species does not seem to be due to competition as a result of eutrophication only, since these species often have already disappeared before grass species become dominant (Houdijk *et al.*, 1993b)

Besides this eutrophicating effect, the enhanced ammonium deposition may lead to acidification of the soil when ammonium is converted to nitrate. Several investigators have already associated the decline of heathland species belonging to the Violion caninae alliance with the acidifying aspect of atmospheric deposition (Van Dam *et al.*, 1986; Fennema, 1990; Houdijk *et al.*, 1993).

Soil acidification may result in a pH decrease, the loss of base cations and release of toxic metal ions (Van Breemen *et al.*, 1982). These effects depend on the pH, buffer capacity and nitrification rate of the soil (Houdijk *et al.*, 1993a). Many of the endangered heathland species of the Violion caninae, Calluno-Genistion, Ericion tetralix and Thero-Airion alliances are found on the less acid (pH 4.2-5.4), slightly buffered sites in heathland soils (Westhoff & Den Held, 1969). Besides pH, the calcium content and the Al/Ca and NH_4/NO_3 ratios were found to be the most discriminating factors between soils on which declining heathland species were able to grow and those with dominant heathland species as dwarfshrubs and several grass species (Houdijk *et al.*, 1993).

In the present study two experiments were conducted to find out whether a pH decrease or increased ammonium levels or NH_4/NO_3 ratios in the soil may account for the decline of rare heathland species. *Thymus serpyllum* L. was chosen as a representative of the endangered heathland species of the weakly acid, slightly buffered heathland soils. The survival of *Thymus* in pot experiments treated with various soil buffering substances in combination with artificial, ammonium containing rain was studied. In a hydroculture experiment nutrient fluxes of *Thymus* in solutions with different NH_4/NO_3 ratios were studied and in an other experiment fluxes of several nutrients of *Thymus* were compared with those of *Calluna* when different forms of nitrogen were offered.

MATERIALS AND METHODS

Pot experiment

Plants of *Thymus serpyllum* were collected in the nature reserve 'De Broekse Wielen' (lat. 51°44'N, long. 5°46'E) in early spring. These plants were carefully removed from the sods and the roots were washed with water. Immediately after this three randomly chosen plants were planted in each of 54 sand containing pots with perforated bottoms (Figure 1). In an open greenhouse (preventing wet and most dry deposition) these plants were sprayed with artificial rain water according to their needs during 6 months (April-October). In the course of the experiment the vitality (as the % green and living parts) of the plants was examined three times.

The sandy soil used in this experiment, originated from De Pannekoele (lat. 52°16'N, long. 6°54'E) and was characterized as moderately acid, slightly buffered and relatively nutrient-poor. One third of the pots (27) contained untreated soil (control) (Figure 1). To the soil of the remaining pots 2.5 g $CaSO_4$/pot (= 19 μM gibsite/g soil) or a combination of 2.5 g $CaSO_4$ and 0.5 g Na_2CO_3/pot (= 6.3 μM soda/g soil) was added. With each of the three differently treated soils 27 pots (ø 10cm) were filled (750 g/pot). In 18 of them *Thymus* was planted (6 replicates per treatment) and to 9 pots artificial rain was added to soils without plants (3 replicates per treatment).

During 6 months all pots were rained with 1.6 l demineralized water containing 25 μM $KNO_3.l^{-1}$ and 5 mg sea salt.l^{-1} to which 0, 250 or 2500 μM $(NH_4)_2SO_4$ was added. In this way 27 pots (18 with plants and 9 without plants) received 0, 15 and 150 kg NH_4-N.$ha^{-1}.yr^{-1}$ respectively.

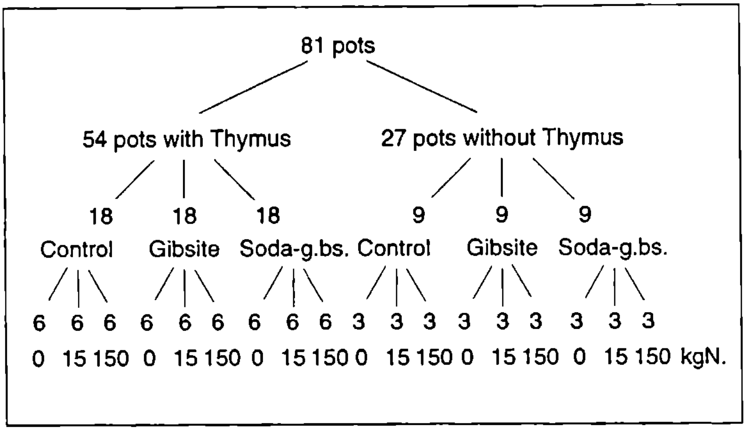

Figure 1: Schematic presentation of experimental set-up.

In order to collect percolation water the pots were placed on blackened plastic containers, to which 0.1 ml fixing solution (200 mg $HgCl_2.l^{-1}$) was added. Monthly, the percolation water was sampled by pooling the yield of six or three pots (with and without plants respectively). After collection the pH was measured immediately and the samples were stored at -27 °C until chemical analysis (Houdijk & Roelofs, 1991).

Scheffé's multiple comparison procedure of the General Linear Models (GLM) procedure was used as a test of significance for differences between means (SAS Institute Inc., 1985). This statistical procedure was performed on log-transformed data, since these fitted better to the conditions of normality (Sokal & Rohlf, 1981).

Hydroculture experiments

The *Thymus* and *Calluna* plants used in these experiments were collected in De Broekse Wielen (lat. 51°44'N, long. 5°46'E) and De Bergse heide (lat. 51°38'N, long. 6°01'E) respectively. Cuttings of both plant species were held in a four times diluted Hoagland medium until roots had developed. Then these cuttings were pre-incubated in either 100 µM NH_4Cl (1) or 100 µM NH_4NO_3 (2) containing media for a two week period. Furthermore, these pre-incubation media contained 1 µM $FeCl_3$, 0.7 µM $ZnSO_4$, 0.8 µM $MnCl_2$, 0.2 µM $CuSO_4$, 0.8 µM H_3BO_3, 0.08 µM $(NH_4)6MO_7O_{24}.4H_2O$ and 5 mg sea salt.l^{-1}. After two weeks the cuttings from pre-incubation medium 1 and 2 were transferred to nutrient solutions of experiment 1 and 2. The nutrient solutions of experiment 1 contained 100 µM NO_3 and varying NH_4 concentrations resulting in NH_4/NO_3 ratios of 0 (I), 1 (II), 10 (III) or 25 (IV) in order to study cation uptake and release of *Thymus* in solutions with different NH_4/NO_3 ratios. In experiment 2 uptake and release of nitrogen and potassium of *Thymus* and *Calluna* when offered different nitrogen forms were compared. The nutrient solutions of this experiment contained 100 µM NO_3 (A), 100 µM NH_4NO_3 (B), 100 µM NH_4 (C) (Table 1). By addition of HCl or NaOH the pH of the nutrient solution was brought to 4.7. Experiment 1 was carried out with cuttings of *Thymus* and experiment 2 with those of *Thymus* and *Calluna*. Each plant cutting was placed on a glass tube (25 ml) filled with nutrient solution so that its roots were completely immersed. The tubes were continuously shaken to prevent oxygen deficiency and nutrient depletion in the rhizosphere. All experiments were carried out in duplo. Nutrient fluxes were calculated from the difference in nutrient concentrations in the experimental media between the start and after 8 or 24 hours in experiment 1 and 2 respectively. After removal of the cuttings the samples were fixed with $HgCl_2$, pH was measured and within 24 hours after terminating the experiment the samples were analyzed. Chemical analyses were performed according to Houdijk & Roelofs (1991). At the end of the experiments dry weight of the plant cuttings was determined in order to estimate the nutrient fluxes per g DW.

Table 1: Chemical composition of the nutrient solutions (in $\mu mol.l^{-1}$) of hydroculture experiment 1 (I, II, III and IV) and hydroculture experiment 2 (A,B and C). I:NH_4 0 NO_3 100; II: NH_4 100 NO_3 100; III:NH_4 1000 NO_3 100; IV: NH_4 2500 NO_3 100; A: NH_4 0 NO_3 100; B: NH_4 100 NO_3 100; C: NH_4 100 NO_3 0.

	experiment 1				experiment 2		
nutrient solution	I	II	III	IV	A	B	C
NH4Cl	-	-	900	2400	-	-	100
KNO3	100	-	-	-	100	-	-
NH4NO3	-	100	100	100	-	100	-
KH_2PO_4	-	100	100	100	-	100	100
NaH2PO4	100	-	-	-	100	-	-
CaCl2	100	100	100	100	100	100	100
MgCl2	100	100	100	100	25	25	25
Na2SO4	-	-	-	-	150	150	150
NaCl	2400	2400	1000	-	350	350	350

RESULTS

Pot experiment

Percolation water of soils without plants contained significantly more NH_4, NO_3, K, Mg, Ca and Al and Al/Ca ratios were higher than those of the soils with *Thymus*. No significant differences were found for pH and NH_4/NO_3 ratio (data not shown).

In Figure 2 the pH of the percolation water of pots with plants and mean vitality (6 replicates) of the *Thymus* plants are presented. In the control soil at all N-treatments the pH of the percolation water decreased during the first four months. In the control soils treated with 150 kg NH_4-N this decrease was, though not significantly, larger (Table 2-I). After four months the pH seemed to stabilize at pH 5.0 and 4.3 in pots treated with 0 and 15 kg NH_4-N respectively. In those receiving the highest N load the pH decreased the first 4 months to 3.4 and, when the vitality had decreased severely, it rose again to 4.1. During the course of the experiment *Thymus* planted on the control and gibsite-soils remained vital at low (0 and 15 kg NH_4-N) deposition. At high deposition the vitality of the plants on the control soil decreased severely whereas on the gibsite-soil *Thymus* declined even faster and died almost completely. The percolation water pH of the gibsite-soil varied little during the experiment, irrespective of N load. The percolation water pH of the soda-gibsite-soils remained high and vitality of *Thymus* was very low during all 6 months when treated with 0 and 15 kg NH_4-N. At high N load the pH decreased rapidly after 3 months and concomittantly the vitality increased after an initial decrease.

Percolation water of all soil types with *Thymus* contained similar amounts of NH_4 after treatment with 0 or 15 kg NH_4-N (Table 2-I). In the control soil the NH_4/NO_3 ratio was somewhat higher after the 15 kg NH_4-N than after 0 kg NH_4-N due to lower NO_3 concentrations. At high N load (150 kg NH_4-N) the NH_4 content and NH_4/NO_3 ratio were significantly higher than at lower loads. In the gibsite and gibsite-soda-soils however, the NH_4 as well as NO_3 content of the percolation water were significantly higher after high N-load but this did not lead to significantly higher NH_4/NO_3 ratios. Average aluminium contents hardly varied with N treatment. Only in control soil aluminium contents were significantly lower at 0 kg than at the 150 kg NH_4-N treatment (Table 2-I). In none of the soil types calcium contents in percolation water showed significant differences between N treatments.

At all N treatments pH of the percolation water and NO_3 contents of the gibsite-soda-soil were significantly higher than those of the control and gibsite-soils (Table 2-II). At none of the N treatments soil type did affect the NH_4 contents of percolation water. Consequently the NH_4/NO_3 ratios at 15 and 150 kg NH_4-N were lowest in the gibsite-soda-soil. At a load of 0 and 15 kg NH_4-N percolation water

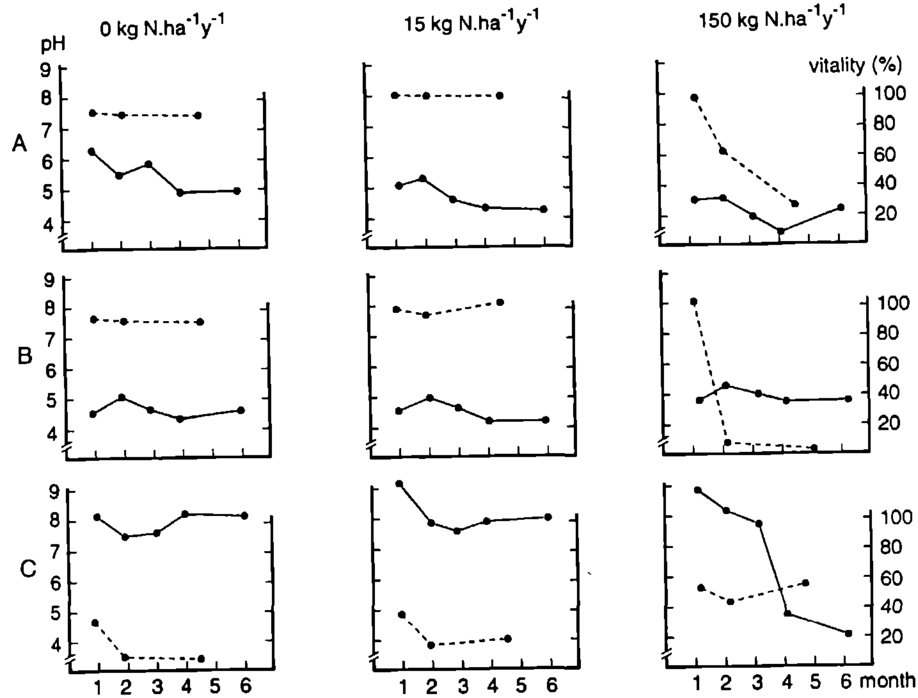

Figure 2: Percolation water pH and vitality of *Thymus* plants on control, gibsite and gibsite-soda-soil during 6 months spraying with articial rainwater containing 0, 15 and 150 kg $NH_4.ha^{-1}.yr^{-1}$. A: control soil; B: gibsite soil; C: soda-gibsite soil; — : pH; - - : vitality.

from the gibsite soil showed significantly higher aluminium contents than both other soil types. At all N treatments calcium contents were significantly lower in the control soil.

Al/Ca ratios in percolation water of all soil types and at all N treatments were very low. Even in the control soil this ratio did not exceed 0.3.

Hydroculture experiments

In Table 3 cation fluxes of *Thymus* in nutrient solutions with different NH_4/NO_3 ratios are shown 8 hours after the start of experiment 1. Nutritional conditions for *Thymus* seemed to be more favourable after pre-incubation in NO_3 containing medium than in a medium without NO_3. However, independent of the pre-incubation

Table 2: Mean pH, NH_4, NO_3, Al and Ca content ($\mu mol.l^{-1}$) and NH_4/NO_3 ratios (mol/mol) in percolation water of three soil types with *Thymus* treated with 0, 15 or 150 kg $N.kg^{-1}.yr^{-1}$. Different letters within a row per soil type (I) or per N-treatment (II) indicate statistical differences at the 5 % level according to Scheffé's. multiple comparison procedure. 0: 0 kg $N.ha^{-1}.yr^{-1}$; 15:15 kg $N.ha^{-1}.yr^{-1}$; 150:150 kg $N.ha^{-1}.yr^{-1}$; A: control soil; B: gibsite soil; C: gibsite-soda soil.

I soil type	A			B			C		
N-treatment	0	15	150	0	15	150	0	15	150
pH	5.8a	5.0a	4.5a	4.7a	4.5a	4.8a	7.7a	8.0a	6.8a
NH4	56b	49b	10661a	48b	62b	15993a	143b	116b	3004a
NO3	92a	4b	85a	5b	2b	182a	52b	85b	6665a
NH4/NO3	1b	14ab	97a	27a	36a	93a	2a	1a	2a
Al	2b	7ab	152a	23a	62a	27a	3a	7a	49a
Ca	547a	861a	1669a	11342a	10081a	8582a	2748b	7458a	9425a

II N-treatment	0			15			150		
soil type	A	B	C	A	B	C	A	B	C
pH	5.8b	4.7c	7.7a	5.0b	4.5b	8.0a	4.5b	4.8ab	6.8a
NH4	56a	48a	143a	49a	62a	116a	10661a	15993a	3004a
NO3	92a	5b	52a	4b	2b	85a	85b	182ab	6665a
NH4/NO3	1b	27a	2b	14a	36a	1b	97a	93a	2b
Al	2b	23a	3b	7b	62a	7b	152a	27a	49a
Ca	547b	11342a	2748b	861b	10081a	7458a	1669b	8582a	9425a

medium cation uptake decreased or release increased with rising NH_4/NO_3 ratios in the nutrient solutions. Cation uptake, particularly K-uptake, was highest in the absence of NH_4.

Table 3: Mean values and standard errors between parentheses of cation fluxes of *Thymus* ($\mu mol.g^{-1}$ DW roots) 8 hours after the start of hydroculture experiment 1. + =uptake, - =release.

nutrient solution	K		Mg		Ca		pre-incubation medium
I NH_4 0 NO_3 100	+9	(1)	+1	(0)	+3	(2)	
II NH_4 100 NO_3 100	+6	(1)	-2	(0)	+1	(1)	NH_4NO_3
III NH_4 1000 NO_3 100	-5	(1)	-2	(0)	-1	(2)	
IV NH_4 2500 NO_3 100	-8	(2)	-2	(1)	-2	(0)	
I NH_4 0 NO_3 100	+12	(2)	0	(3)	+1	(2)	
II NH_4 100 NO_3 100	+1	(5)	-4	(1)	-3	(4)	NH_4Cl
III NH_4 1000 NO_3 100	-8	(1)	-6	(2)	-15	(8)	
IV NH_4 2500 NO_3 100	-4	(2)	-2	(1)	-2	(0)	

Table 4: Mean values and standard errors between parentheses nitrogen and potassium fluxes of *Thymus* and *Calluna* (µmol.g^{-1} DW plants) 24 hours after the start of hydroculture experiment 2. + =uptake, - =release.

nutrient solution		NH_4		NO_3		K		pre-incubation medium
Thymus								
A NH_4 0	NO_3 100	+1	(0)	+20	(12)	+17	(10)	
B NH_4 100	NO_3 100	+32	(6)	+17	(4)	+2	(4)	NH_4NO_3
C NH_4 100	NO_3 0	+49	(8)	0	(0)	+8	(2)	
A NH_4 0	NO_3 100	+1	(0)	+24	(1)	+21	(1)	
B NH_4 100	NO_3 100	+16	(3)	+7	(3)	+1	(3)	NH_4Cl
C NH_4 100	NO_3 0	+30	(2)	0	(0)	+3	(1)	
Calluna								
A NH_4 0	NO_3 100	0	(0)	-4	(4)	-1	(0)	
B NH_4 100	NO_3 100	+3	(3)	-1	(0)	-2	(3)	NH_4NO_3
C NH_4 100	NO_3 0	+14	(1)	-1	(0)	-1	(1)	
A NH_4 0	NO_3 100	0	(1)	-10	(3)	-4	(1)	
B NH_4 100	NO_3 100	+12	(4)	-4	(1)	+5	(2)	NH_4Cl
C NH_4 100	NO_3 0	+15	(15)	-1	(1)	+7	(6)	

In Table 4 NH_4, NO_3 and K fluxes of *Thymus* and *Calluna* are shown 24 hours after the start of experiment 2. Ammonium inhibited nitrate uptake by *Thymus*. K uptake by *Thymus* was highest in the NO_3 containing medium and was reduced in the presence of NH_4. The pre-incubation medium hardly affected the K uptake by *Thymus*.

In contrast to *Thymus*, *Calluna* did not take up any nitrate. Both ammonium and potassium uptake by *Calluna* were highest in the NH_4 containing solution after NH_4Cl pre-incubation. Pre-incubation in a NO_3 containing medium seemed to affect K-uptake by *Calluna*.

DISCUSSION

Effects of pH

Thymus serpyllum generally grows on soils within a pH range of 5.0 to 5.5 whereas the dominating heathland species as *Calluna*, *Erica* and *Molinia* occupy the more acidic soils within a range of 3.9 to 4.2 (Houdijk *et al.*, 1993b). This discrepancy led to the assumption that the decline of *Thymus* and many other herbaceous heathland species that are bound to less acidic, weakly buffered heathland soils might be attributed to soil acidification as a result of atmospheric deposition (Van Dam *et al.*, 1986; Fennema, 1990; Houdijk *et al.*, 1993b).

The results of the pot experiment, however, indicated that *Thymus* is able to survive at low pH values. In this experiment, although percolation water pH frequently reached pH values far below its field range, *Thymus* plants remained vital. Van Dobben (1991) found in hydroculture experiments that indeed optimum pH values of several declining species (among others *Arnica montana* L. and *Gentiana pneumonanthe* L.) are higher than those of several dominating heathland species. However, the declining species were able to survive within a very wide pH range. The pH values of the lower limit of this range were as low as those of the dominating species. Furthermore, this pot experiment indeed indicated the existence of an upper limit for this pH-range as the vitality of *Thymus* severely decreased at high pH values.

Indirectly soil acidification affects the composition of the exchangeable cations in the soil. Houdijk *et al.* (1993b) found that in the soil of *Thymus* and many other endangered heathland species Al/Ca ratios were clearly lower than in the soil of *Calluna*, *Erica* and *Molinia.* Acid loads on the soil of the endangered heathland species will initially be buffered by cation-exchange processes. When eventually calcium becomes exhausted and pH drops to 4.2 aluminium mobilization and dissolution will gradually become more important (Ulrich, 1983). Not only increased aluminium concentrations are harmful to plant roots (Hütterman, 1985) and inhibit calcium and magnesium uptake (Evers, 1983), but, according to Ulrich, also enhanced Al/Ca ratios (>5) may cause root damage, Boxman *et al.* (1991) found that young coniferous trees on hydroculture were already damaged at an Al/Ca ratio of 1.

In the pot experiment in the gibsite-soil treated with 15 kg NH_4-N and in the control and gibsite-soda soil treated with 150 kg NH_4-N pH values below 4.2 were reached. Then indeed in these soils aluminium concentrations in the percolation water were higher than in percolation water of all other treatments. Comparison of the vitality on all soil types treated with the highest N-load indicates that these high Al levels do not account for the decline of Thymus. This may be due to the fact that in this experiment Al/Ca ratios never reached critical levels due to the fact that also calcium levels were increased. However, in hydroculture experiments vitality of *Arnica* severely decreased when incubated at 100 µmol aluminium irrespective of calcium concentration (De Graaf, pers. comm.). Maessen *et al.* (1992) found that high calcium concentration has a counteracting effect on the toxicity of aluminium for several aquatic plant species.

Gigon & Rorison (1972) found that aluminium toxicity depends on the type of nitrogen: nitrate uptake enhances the pH of the root zone and thus reduces solubility of aluminium, while ammonium uptake decrease the pH of the root zone. However hydroculture experiments proved that plants respond in various ways to sources of nitrogen and that aluminium toxicity depends on the preference of those plants for different nitrogen sources (Rorison, 1985; 1986). Species of acid soils like *Deschampsia flexuosa* are able to grow on nitrate as well as on ammonium in the presence of aluminium at low pH while generally species that grow on less acidic soils prefer nitrate and are more sensitive to aluminium. *Thymus* seemed to benefit more from nitrate nutrition, but was also able to take up ammonium, however, the results of this experiment are not conclusive about the relation between nitrogen source and aluminium toxicity.

Effects of nitrogen

In the Netherlands soil acidification is mainly due to enhanced atmospheric deposition of ammonium as a result of nitrification. Roelofs *et al* (1985) found that in heathland soils this process is inhibited at pH 4.2 or lower. In these soils the transformation rate of ammonium to nitrate is not high enough to prevent ammonium accumulation, resulting in increased NH_4 levels and NH_4/NO_3 ratios in the soil solution. The soil pH of the declining heathland species is generally higher than 4.2. In these soils low NH_4/NO_3 ratios indicated that nitrification still takes place (Houdijk *et al.*, 1993b). The pot experiment showed that at low ammonium deposition the pH of percolation water of the control and gibsite-soils initially decreased and then remained stable. Based on the NH_4/NO_3 ratios it can be derived that during the course of the experiment nitrification took place at very low rates. In the soils treated with gibsite plus soda, lower NH_4/NO_3 ratios indicate higher nitrification rates. At very high ammonium load and low pH, nitrification rates in the control and gibsite-soil were not large enough to prevent ammonium accumulation and resulted in high NH_4 levels and NH_4/NO_3 ratios in the percolation water. In these situation the vitality of *Thymus* clearly decreased. In the gibsite-soda-soil nitrification rates were much higher due to high soil pH and resulted in low NH_4/NO_3 ratios and eventually in a lower soil pH. Despite very high nitrogen concentrations *Thymus* is vital in this situation. These findings indicate that *Thymus* is able to survive very high ammonium loads as long as nitrification rates are high enough to balance ammonium and nitrate. The importance of the presence of nitrate is emphasized in hydroculture experiments. Potassium uptake was clearly stimulated in the presence of nitrate. In contrast to *Thymus*, *Calluna* , pre-treated in a NH_4 containing nutrient solution, took up K from experimental media containing NH_4 or NH_4NO_3 while uptake was completely inhibited when only NO_3 was available in the experimental medium. *Calluna* plants pre-treated in NH_4NO_3 containing media showed no K uptake at all, irrespective whether NH_4, NO_3 or NH_4NO_3 was available in the experimental media.

Conclusions

Though *Thymus* plants are generally found to grow on soil with relatively high pH, pot experiments showed that they are able to survive on soils with low pH. The pH decrease as a result of the enhanced ammonium deposition does not seem to be directly responsible for the decline of this species. However, this pot experiment indicated that unbalanced amounts of NH_4 and NO_3 rather than the total amount of nitrogen may account for the decline of *Thymus*. These findings are confirmed by the results of hydroculture experiments which clearly demonstrated that the cation nutrition of *Thymus* benefited from the presence of NO_3, whereas *Calluna* was more adapted to ammonium nutrition.

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CHAPTER 9

GENERAL DISCUSSION

In the previous chapters the decline of two terrestrial ecosystems in the Netherlands in relation to the enhanced ammonium deposition has been described. It has been mentioned in the first chapter that heathland vegetation and coniferous tree species grow under nutrient poor, weakly buffered and acidic conditions and, therefore, are susceptible to eutrophication as well as acidification. In this chapter the main conclusions will be discussed and the similarity of the problems that affect these ecosytems will be emphasized.

Quantification of atmospheric deposition of pollutants is of great importance to understand the impact of these pollutants on ecosystems. Evidently throughfall fluxes do not equal atmospheric deposition fluxes as neither canopy exchange nor stemflow are measured in throughfall samples. However, for elements that are deposited at very high rates such as sulphuric and nitrogenous compounds, throughfall fluxes can be considered equivalent to total atmospheric deposition (Bredemeier, 1988; Ivens, 1990).

All forested areas in The Netherlands appear to receive high fluxes of pollutants, in particular of ammonium and sulphate (chapter 3). Only in the coastal area lower throughfall fluxes have been measured. Ammonium pollution has proved to be no longer a regional problem, confined to areas with intensive husbandry. Since the acceptance of the role of ammonia or ammonium as a major pollutant the emission sources initially increased due to expansion of livestock farms but also to the extreme expansion of liquid-manured arable land in the Netherlands and abroad (Asman, 1987). Research into the atmospheric behaviour of reduced nitrogen compounds also aided in proving that these pollutants can be transported over a long distance and are not only deposited in the neighbourhood of the ground-leveled emission sources (Asman, 1987). However, despite elaborate studies there still exists a discrepancy between measured (throughfall method) and calculated dry deposition fluxes. The latter are generally lower leading to an underestimation of the contribution of reduced nitrogen compounds to total atmospheric deposition. This difference can be explained by the fact that in calculations co-deposition of ammonium and sulphate is not taken into account (Duyzer *et al.*, 1988). The spatial variation of deposition fluxes within a forested area is very large. Consequently, it is very difficult to estimate average transformation, turbulence and deposition rates necessary for the calculation of atmospheric deposition fluxes.

Moreover, the collection of throughfall water has proved to be an adequate method to measure the actual load of acidifying and eutrophicating substances onto the forest floor (chapter 2 and 3, Ivens, 1990).

The scavenging action of tree canopies leads to extremely high deposition fluxes on the forest floor, about 75 % of potential acid (7.2 kmol $H^+.ha^{-1}.yr^{-1}$ is deposited in a dry form (chapter 3). On an average sulphate contributes 46 %, ammonium 44 % and nitrate 10 % to the load of potential acid in Dutch forests. Only the contributions in the relatively unpolluted coastal area deviate from these average values. In this region nitrate and sulphate contributions are larger, 55 % and 16 % respectively.

Generally forest ecosystems are nitrogen limited and nitrogen fertilization easily leads to enhanced nitrogen uptake and biomass increment. In relatively unpolluted areas abroad throughfall fluxes of nitrogen compounds are frequently lower than bulk fluxes indicating nitrogen uptake by the canopy. In Dutch forests exchange of base cations with ammonium in the canopy may account for the nutrient deficiencies often observed in trees of decreased vitality. The high deposition rates of ammonium in Dutch forests probably obscure the uptake rates whereas too little is known yet about the deposition rates of the exchanged cations. Ecophysiological experiments with Corsican pine indeed proved extensive ammonium uptake by needles and potassium, magnesium and calcium release when they were incubated in ammonium containing medium (chapter 2). Uptake of ammonium by the canopy can explain the high nitrogen levels and low cation levels measured in damaged trees (chapter 2) and may account for the positive correlation between the ammonium deposition and nitrogen content of needles in pine forests (chapter 5).

However, nutrient uptake by the roots evidently plays a major role. The field investigation discussed in chapter 2 clearly demonstrates a correlation between the mineral soil balance and the condition of trees. High ammonium/potassium and ammonium/magnesium ratios were often measured in soils of less vital forests whereas healthy forests were generally found on soils with lower ratios. Consequently, critical values for these ratios are established at 5 and 10 respectively. Nation-wide research in Dutch coniferous forests has shown that a strong correlation exists between ammonium fluxes and the ratios of ammonium to cations in deposition at one hand and ammonium content and ammonium/cation ratios in the upper soil layer on the other hand (chapter 4). The ratios in soils with low nitrification rate show usually the best agreement with those in the deposition. In this study estimates of nitrification rates have shown that in 67 % of the forest soils this rate was probably very low. Even in the stands with higher nitrification rates ammonium remained the dominant compound of inorganic nitrogen in the top 15 cm of the soil.

The nutritional status of needles of Douglas fir, Scots pine and Corsican pine has been clearly and most probably indirectly affected by the high ammonium fluxes in the deposition, via the soil compartment. This indirect relation not only has become apparent from the stronger correlations between deposition fluxes and soil composition, but also from the correlations between deposition fluxes and needle composition (chapter 5). The results of a greenhouse experiment have confirmed that uptake of ammonium by the canopy is probably negligible (Van Dijk *et al.*, 1990). Preliminary results of reduced atmospheric nitrogen and sulphur deposition on the nutritional status of soil and needles also indicate the indirect effect of ammonium deposition (Van Dijk *et al.*, 1992b).

The field investigations discussed in chapter 2,3 4 and 5 have provided information on the relation between atmospheric deposition and the nutritional status of the forest ecosystem. The nutritional disharmony of trees and soil caused by the enhanced ammonium deposition is now generally accepted as an explanation to the forest dieback in Europe (Nihlgård, 1985; Van Dijk *et al.*, 1989, 1990; Schulze *et al.*, 1989). The soil percolation experiments discussed in chapter 5 have been conducted to provide the information on the causality of the observed relation between the composition of deposition and soil which is needed for the estimation of the critical nitrogen load on forest soils. For this purpose the threshold values of the ammonium/potassium ratio (estimated to be 5, see chapter 2) and the aluminium/calcium ratio in the soil (estimated to be 1 according to Ulrich (1983)) have been used as indicators for the nutritional disbalance.

Evidently critical ammonium deposition levels vary for different soil types. Particularly the nitrification rate of the forest soil type seems to determine the kind of disbalance. In highly nitrifying soils acidification as a result of the conversion of ammonium to nitrate leads to aluminium mobilisation and base cation leaching and consequently to raised Al/Ca ratios. In lowly to moderately nitrifying soils the exceedance of the threshold value of the NH4/K ratio determines the critical deposition level which is mainly due to ammonium accumulation. Two third of the soils of coniferous stands show low nitrification rates which lead to high ammonium levels in the rooted top layer (chapter 4) indicating that in most of these soils the eutrophicating rather than the acidifying impact of ammonium causes the nutritional disbalance.

Naturally, besides nitrification rate also base saturation of the soil and the nutritional status of trees are of importance. However, generally base cation content of coniferous forest soils is low. Most of them have low pH values (4.2) and acid is neutralized by aluminium (aluminium buffer range). Furthermore, probably most Dutch forest stands are no longer nitrogen-limited. Nitrogen application no longer leads to increased biomass production and many trees in the Netherlands show absolute as well as relative high nitrogen contents in the needles (chapter 2, 5; Van Dijk *et al.*, 1988, 1992a). The mineral balance in the columns filled with soil of nitrogen saturated forests also does not change when the amount of ammonium added is similar to the amount the soils received in the field. This emphasizes the fact that the absence of vegetation is hardly of any effect when using soil percolation columns in determining the critical nitrogen load. Clearly, this critical level depends on the most sensitive soil type and generally this will be the soil with the lowest base saturation and nitrification rate.

In heathland soils the enhanced ammonium deposition seems to cause very similar problems such as in the forest soils. Soil processes such as acidification, ammonium accumulation, nitrification and aluminium mobilization determine the soil quality and consequently the species composition of the heathlands (chapter 7). The most important differentiating process again appears to be the nitrification process that, despite the low pH (pH 4) values, may take place in heathland soils (De Boer, 1989). Roelofs *et al.* (1984) have found that total nitrogen levels in grass dominated heathland are much higher than in heather dominated heathlands. In both heathland types ammonium levels are 10-20 times higher than those of nitrate. However, in heather dominated heathland hardly any nitrate can be measured. De Boer (1989) has found that in soils with young dwarfshrubs no nitrate production takes place whereas in older dwarfshrub vegetation and grasslands nitrate is produced. He suggests that there may be a relation between the start of nitrate production in heather dominated heathlands and the requirement of management, in order to prevent the transition to grass dominated heathlands. He also has found that nitrate production in heathlands may be limited by the ammonium availability. In contrast, by Roelofs (1986) and in chapter 2 it has been argued that the accumulation of ammonium due to the inhibited nitrate production at low pH values in dwarfshrub dominated heathland soils accounts for the transition to grass dominated heathlands. However, both mechanisms agree with the general acceptance that the change-over from dwarfshrub dominated to grass dominated heathland is due to higher nitrogen, in particular the ammonium availability from which grass species are able to benefit more than heather species. In chapter 7 it is stated that the decline of rare herbaceous heathland species can not entirely be due to the increased nitrogen availability since they are not only taken over by grasses but also by dwarfshrub species; further, nitrogen levels in the soil of the rare species are similar to those of the heather species. However, pH values and calcium levels are generally higher and the Al/Ca ratios are lower in the soil of the rare species than in the soil of the dominant (dwarfshrubs or grasses) species whereas ammonium/nitrate ratios are of intermediate value. These findings lead to the assumption that the decline and disappearance of these rare species might be due to the acidifying effect of ammonium deposition rather than to its eutrophicating effect (chapter 7, Fennema, 1990). This can either be due to the pH decrease itself or by acidification mediated processes, such as loss of base cations, increased aluminium dissolution or inhibited nitrification. In chapter 8 experiments with *Thymus serpyllum* L. reveal that neither a pH decrease as a result of the enhanced ammonium deposition nor the total amount of nitrogen seem responsible for the decline of this species. However, they indicate that the decline may be due to the unbalanced amounts of ammonium and nitrate when as a result of the lower nitrification rates at lower pH values less nitrate is produced (see also chapter 2). Furthermore, it is indicated that the unbenificial cation nutrition of *Thymus serpyllum* in the presence of ammonium probably accounts for this phenomenon. However, preliminary results of research with *Arnica montana* L. show that increased aluminium levels probably also contribute to the decline of these species (pers. comm. De Graaf). Moreover, it is quite well possible that the various heathland species react in different ways to the various components of the environmental deterioration, as a consequence of nitrogen deposition, the one being more susceptible to pH, the other to aluminium or to the ammonium/nitrate ratio (chapter 7).

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SAMENVATTING

In dit proefschrift worden de resultaten van een onderzoek naar de relatie tussen atmosferische stikstofdepositie, in het bijzonder die van ammonium, en de degeneratie van terrestrische ecosystemen besproken. In Nederland worden de meeste naaldbossen en heidevegetaties aangetroffen op de zwak-gebufferde, kalk- en nutriënten-arme zandgronden. Deze bodems blijken zeer gevoelig voor de verzurende en eutrofiërende effecten van atmosferische depositie.

Een van de eerste studies waarin deze relatie werd onderzocht beperkte zich tot naaldboomopstanden in het zuid-oostelijk deel van Nederland (hoofdstuk 2). In deze zogenaamde Peel-regio kon men in het begin van de jaren tachtig een van de grootste concentraties intensieve-veehouderijbedrijven aantreffen. In dit onderzoek werd vastgesteld dat de depositie van ammoniumsulfaat in de nabijheid van deze ammoniak-emissiebronnen extreem hoog is. De conditie van naaldbossen in deze regio bleek opvallend slecht en in naalden van aangetaste Corsicaanse dennen (*Pinus nigra*) werden lage kalium- en magnesiumgehalten gemeten. In laboratoriumexperimenten werd de samenhang tussen ammoniumdepositie en lage nutriëntengehalten bevestigd. Zo bleek dat naalden in staat zijn ammonium uit te wisselen tegen kalium, magnesium en calcium. Daarnaast bleken deze elementen onder invloed van ammonium, uit de bodem te spoelen waardoor de nutriëntenopname door de wortels wordt belemmerd. Verstoring van de nutriëntenhuishouding in de bodem en het optreden van deficiëntieverschijnselen in de vegetatie bleken sterk afhankelijk van de bodemsamenstelling. Met name de verhoogde ammonium/kation-ratio's in de bodem vertoonden een sterke correlatie met de vitaliteit van de dennen.

Een uitgebreider onderzoek naar de samenstelling van de atmosferische depositie wees uit dat in naaldbossen verspreid over heel Nederland de kritieke waarden van de potentiële zuur- en de totale stikstofdepositie worden overschreden (hoofdstuk 3). Uit metingen van doorval- en bulkfluxen bleek dat in de kustregio de atmosferische depositie lager is dan in de overige regio's, maar zelfs hier worden de depositienormen ruimschoots overschreden. Ook de samenstelling van de depositie week hier af door een lagere bijdrage van ammonium aan de potentiële zuur- en totale stikstofdepositie. De ruimtelijke variatie in depositie binnen en tussen de overige regio's bleek voornamelijk toe te schrijven aan de verschillen in bosstructuur.

Uit het landelijk correlatieve onderzoek is tevens gebleken dat de hoge stikstofdepositie in de vorm van ammonium direct verantwoordelijk is voor de verstoorde mineralenbalans in de bodem (hoofdstuk 4). De mate van verstoring werd bepaald aan de hand van de verhouding van ammonium en basische kationen of die van aluminium en calcium. Hoge ratio's duiden op een onevenwichtige voedingsstoffenbalans en bleken vaak gecorreleerd met een slechte bosconditie (hoofdstuk 2). Met name in bodems met beperkte nitrificatie (67 % van de onderzochte locaties) werd een sterke relatie tussen de ammonium/kation-ratio's in depositie en bodem gevonden.

Beregeningsexperimenten met bodemkolommen bevestigden deze veldwaarnemingen (hoofdstuk 6). De afname of toename van de hoeveelheid toegediende ammonium ten opzichte van de hoeveelheid ammonium in de velddepositie bleek te leiden tot een afname respectievelijk een toename in de mate van verstoring van de mineralenbalans in de bodemcores. In bodems met een beperkte nitrificatiesnelheid bleek dat de kritieke waarde van de ammonium/kalium-ratio al bij lage ammoniumdepositie bereikt werd. In bodems waar ammonium snel wordt omgezet in nitraat trad verstoring van de mineralenbalans op bij hogere depositie, in dit geval meestal door overschrijding van de kritieke waarde van de aluminium/calcium-ratio.

In het landelijk onderzoek werden op elke locatie, naast depositie- en bodemmonsters, ook naaldmonsters verzameld (hoofdstuk 5). Zowel de absolute als relatieve nutriëntengehalten in naalden van Corsicaanse den (*Pinus nigra*), grove den (*Pinus sylvestris*) en Douglas spar (*Pseudotsuga menziesii*) bleken beïnvloed te worden door de hoge ammoniumdepositie, waarschijnlijk via het bodemcompartiment. Absoluut gezien bleek fosfor het meest kritische element: in 90% van de sparren en 40% van de dennen werd een te laag fosforgehalte gemeten. Relatieve magnesiumdeficiëntie bleek vooral vaak op te treden bij dennen (80%) en in mindere mate bij de spar (30%). Het verschil in de chemische samenstelling tussen naalden van spar en dennen hangt waarschijnlijk samen met hun voorkeur voor nitraat respectievelijk ammonium als stikstofbron.

In de hoofdstukken 7 en 8 wordt de achteruitgang van enkele bedreigde, kruidachtige planten van het heidemilieu besproken. De bodem waarop deze planten voorkomen verschilt vooral in pH, aluminium/calcium-ratio en in mindere mate in ammonium/nitraat-ratio van bodems waarop de dominante heidesoorten (dop- en struikheide) en grassoorten (pijpestrootje) groeien. (hoofdstuk 7). Uit dit onderzoek kan worden afgeleid dat het verdwijnen van deze bedreigde soorten mede is toe te schrijven aan de verzurende effecten van de ammoniumdepositie en niet primair aan de eutrofiërende effecten.

Experimenten met *Thymus serpyllum* bevestigden de relatie tussen de achteruitgang en het verzurende effect van ammonium (hoofdstuk 8). Hieruit bleek echter dat de pH-daling als gevolg van een verhoogde ammoniumdepositie waarschijnlijk niet de oorzaak is voor het verdwijnen van deze soort uit de heideterreinen. Ook bleek uit potproeven dat deze soort kon overleven bij een hoge stikstofconcentratie, echter uitsluitend daar waar stikstof voor een belangrijk deel in de vorm van nitraat voorkomt. Uit aanvullende experimenten bleek dat deze soort inderdaad gevoelig is voor verhoogde ammonium/nitraat-ratio's waarbij de kationhuishouding verstoord raakt.

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CURRICULUM VITAE

Anneke Houdijk werd geboren op 29 augustus 1960 te 's-Hertogenbosch. Na het behalen van het diploma Atheneum B aan het St. Janslyceum te 's-Hertogenbosch in 1978 begon zij in datzelfde jaar de studie Biologie (B1g) aan de Katholieke Universiteit te Nijmegen. Tijdens de doctoraalfase specialiseerde zij zich in de vakken Aquatische Oecologie (hoofdrichting), Biogeologie en Milieugeografie (nevenrichtingen). Daarnaast werd de cursus Didactiek van de Biologie gevolgd. In februari 1986 behaalde zij het doctoraalexamen (met genoegen). Van augustus 1986 tot mei 1992 was zij als toegevoegd onderzoeker werkzaam aan de Katholieke Universiteit te Nijmegen bij de Vakgroep Aquatische Oecologie en Biogeologie, thans Werkgroep Milieubiologie van de Vakgroep Oecologie. In opdracht van het Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer werd een drie-jarig onderzoek uitgevoerd naar de effecten van zwavel- en stikstofdepositie op bos- en heidevegetaties. Een groot deel van de resultaten van deze studie zijn verwerkt in dit proefschrift. Aansluitend werden gedurende twee en een half jaar, wederom in opdracht van VROM, de effecten van verhoogde aluminium gehalten in de bodem op de vitaliteit van Nederlandse bossen onderzocht.